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FRANK LINCOLN STEVENS

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(WITH PORTRAIT)

Frank Lincoln Stevens,[1] Professor of Plant Pathology in the University of Illinois, died August 18, 1934, in his sixty-third year, at Winnetka, Illinois. He had held a preeminent place among mycologists for two decades and had been a prominent, active, and productive personage in American plant pathology for nearly thirty-five years. His premature death ended a life exceptionally useful in his chosen fields and as a teacher, the excellence and sincerity of whose instruction have found expression with gratifying frequency in the achievements of his students. He was highly regarded in America and much honored abroad, and his passing has left a conspicuous gap in the ranks of those devoted to applied and technical mycology.

Descended from a pioneer family of Onondaga County, New York, the environment of his early years, spent on a farm near Syracuse, fostered the development of that inborn love of nature which was, throughout his life, the inspiration of his accomplishments. As a youth, he made a comprehensive collection of the ferns of Onondaga County and also a representative collection

[1] Biographical data are given in both Who's Who in America and American Men of Science. Space does not permit printing the long list of articles and books written by F. L. Stevens.

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of geological specimens from the same region; and he equipped for himself, at home, a laboratory in which, with only the aid of manuals and texts, he obtained such a mastery of chemistry as to be able to pass examinations and to graduate from both high school and college with chemistry courses to his credit, without having had any formal instruction in the subject.

At about the time of his graduation from Hobart College Stevens, while casting about for some phase of natural science to which he could devote himself with a prospect of usefulness and success, went on a visit to the Agricultural Experiment Station at Geneva, New York. There he discussed his problem with David G. Fairchild, who suggested that the field of fungi and plant diseases appeared to offer a splendid opportunity and that he might do well to associate himself for study with Fairchild's uncle, Byron D. Halsted, then pioneering in American plant pathology. Acting on this suggestion Stevens went to Rutgers College, where between 1891 and 1893 he acted as student assistant and in the latter year received the B.S. degree.

Stevens then became teacher of science in Racine College for a year and while there became acquainted with Dr. J. J. Davis, at that time a practicing physician developing the interest in fungi which has since given him a unique position in American mycology. The following year Stevens became teacher of chemistry and botany in the high school at Columbus, Ohio, and it was while holding this position that he received the stimulus which brought him eventually into the broader field of his life's work. Though not formally a student in the University of Ohio, he had been given the privilege of working in the University's laboratories. While satisfying on one occasion his desire to ramble in the woods, he rested on a fallen log and at the moment picked up some leaves attacked by a parasitic fungus. Returning with them to the laboratory, he found them to contain all stages of the developing oöspores of an *Albugo*. The studying of this material began to occupy his time and attention and, after receiving the M.S. degree from Rutgers College in 1897, he went to the University of Chicago where, as fellow in botany, he completed his classical studies of the fertilization and development of the oöspores of *Albugo* and received, for his thesis, the Ph.D. degree, *magna cum laude*, in 1900.

The University of Chicago awarded him a traveling fellowship for the following year and he went first to Bonn and Halle, studying under Strasburger, Klebs, and others, and later to the Naples Zoölogical Laboratories, where he was awarded a table under the auspices of the Smithsonian Institution.

Upon returning from Europe he became, first, instructor in biology, and then professor of botany and plant pathology in the North Carolina College of Agriculture and Mechanic Arts, which position he continued to hold until 1912. During many of these years he was also biologist and head of the department of plant diseases of the North Carolina Agricultural Experiment Station.

The years in North Carolina were noteworthy in Stevens' life for many things subsequently of importance in the science of plant pathology, but the extent of his interest was never limited to pathology alone. It was here that he wrote "Diseases of Economic Plants" with J. G. Hall, the first comprehensive handbook of plant diseases published in America. Here, too, he undertook the study of many problems of fundamental importance in American plant pathology, notable among them control of tobacco diseases by soil sterilization and breeding and selection of cowpeas and watermelons resistant to wilt diseases, and championed the use of the "ose" termination for names of plant diseases. Feeling that school books might be made more vitally interesting to children on farms, he collaborated with others of the college and became the joint author of "Agriculture for Beginners," the "Hill Readers," and a "Practical Arithmetic," books intended to facilitate learning by school children through the use of familiar agricultural facts and illustrations.

In his forty-first year Stevens resigned his position in North Carolina to become Dean of Agriculture in the University of Porto Rico and undertook the organization of the College of Agriculture and Mechanic Arts of that institution. While in Porto Rico his insatiable curiosity caused him to make an extensive collection of Porto Rican fungi, which he brought with him on his return to the United States. This collection became the basis of many important mycological contributions by himself and his students. The manuscript of his best known book, "The Fungi Which Cause Plant Disease," was prepared and sent for publication during his sojourn in Porto Rico.

Upon his return to the United States in 1914 he was appointed Professor of Plant Pathology in the University of Illinois, and this position he maintained until his death. At Illinois, however, his interest gradually turned from plant pathology to mycology, in part because of the development of specialized fields of plant pathology in the Illinois Agricultural College but more particularly because, with his advancing years and increasing maturity, the finely intellectual problems of taxonomic mycology, the importance of which his early associations had emphasized, appealed to him more and more strongly.

While his accomplishments in plant pathology, largely realized before his arrival at the University of Illinois, have given him an outstanding place among early American workers, the full importance of his achievements in mycology are yet to be fully appreciated. As an active collector, he traveled into South America, Panama, Hawaii, and the Philippines, returning from each place with large numbers of originally and personally collected specimens from which he developed pioneer studies of the mycological floras of these regions. These studies and his monographic treatments brought him world-wide renown.

Stevens received many honors at home and abroad. The University of San Marcos, at Lima, conferred upon him in 1925 the Sc.D. degree. On the Illinois campus he was a marked figure in the scholarly lines of professors, when gowns and hoods were in order, because of a brilliant scarlet gown worn in signification of the LL.D. awarded by the University of Glasgow in 1928. He was, during his years in North Carolina, a member of the North Carolina State Board of Examiners for the certification of teachers and, in later years, took much pride in his success as a lecturer at farmers' institutes. He was a Fellow of the American Association for the Advancement of Science, a member of the Wisconsin, Ohio, and Illinois academies of science, president in 1905 of the North Carolina Academy of Science, a member of the American Genetic Association, of the Botanical Society of America, the Society of American Bacteriologists, the American Society of Naturalists, the American Nature Study Society, of which he was vice president in 1908, the American Phytopathological Society, of which he was president in 1910, and the Mycological

Society of America, as well as many others. Since 1910, Stevens' name has been among those starred in American Men of Science.

I find it impossible to close this tribute without a personal word in which all of his students, many of whom are also my friends, may join. He was always unassuming and modest, uniformly courteous and patient, with a tendency to speak little of himself but to be much concerned with the good of others. Yet few, if any, of us ever knew him intimately, for his time and his thoughts were so completely devoted to us, in order that we might get all he had to give, that his own affairs were never in evidence. In a University which gives no degree in plant pathology and in a Department which requires of its students first of all a general proficiency in botany, graduate students interested in all phases of botany came under his instruction, and for years those majoring in his subjects made up the majority of the advanced students in the department. Almost without exception, his students are today holding honorable and influential positions in teaching or research in plant pathology and mycology. Among the 285 charter members of the Mycological Society of America, 10 have been students of his and 8 of these obtained their doctorates under him.

In his teaching he was inspiring and at the same time particular and thorough. While he believed, and sought always to impress his students with the belief, that deliberate efforts to create things of immediate usefulness were almost certain to result in shoddy work and that the project method was the greatest pitfall of research, the mysteries of natural phenomena always presented to him the challenge of a needed solution, and the great variety of subjects that came under his consideration, and to which he endeavored to introduce his students, made association with him stimulating indeed. He made use, not so much of his own knowledge and experience, as of examples and the best of literature. It may be said that in all his teaching he followed the behest of Abel, the mathematician, who explained his own accomplishments briefly by saying he had studied the masters, not the pupils. Himself a master, it was to the inspiration of other masters that Frank Lincoln Stevens sought to lead his pupils.

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SOME CONIDIAL PHYCOMYCETES DESTRUCTIVE TO TERRICOLOUS AMOEBAE

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(WITH PLATES 1–7)

INTRODUCTION

In isolating parasites referable to the genera *Pythium, Phytophthora* and *Aphanomyces* from decaying plant tissues, I have been employing for some years a method which as described earlier (**7**) entails no attempt through surface disinfection at avoiding contamination of the isolation plates by the extraneous organisms habitually present on such material. After serving their immediate purpose, the isolation plates, as might be expected, often come to display a varied intermixture of adventitious plant and animal life, the character of the mixture in each case evidently depending somewhat on the nature and in larger measure on the source of the material used. Subterranean parts like roots, tubers, stolons and basal portions of stems, or even aerial parts like fruits and leaves that have been in contact with the ground, generally yield *Amoebae* of different species, the smaller forms apparently feeding mostly on bacteria, the larger ones ingesting cysts of the smaller *Amoebae,* testaceous protozoans, nematode eggs and more especially fungous spores of all descriptions.

While *Amoebae* in agar cultures are thus revealed as very active in the rather indiscriminate destruction of fungous life, they are in turn subject to wholesale destruction here and presumably also in nature by specific parasitic and predacious fungi. In a previous note (**9**) the morphological features of five fungi predacious on *Amoebae* were set forth, four of these being readily recognizable as Phycomycetes in spite of their Hyphomycete-like aerial conidia and of a vegetative mycelium which in three of the species is comparable in delicacy to the mycelium in species of *Actinomyces.* Several other forms similarly producing non-catenulate conidia and

similarly displaying moderately extended mycelial or haustorial development within the smaller *Amoebae* captured by them, have since been observed. Although the sexual reproductive bodies of one of these additional forms are somewhat larger than those of any of the three for which such bodies were figured earlier (**9**, *figs. 3–5*), they are yet of dimensions too small to provide in themselves entirely decisive evidence as to their exact structure; or, for that matter, in view besides of the little differentiated and hence undistinctive character of the fusing elements, as to their correct interpretation. In referring earlier to the ambiguous sexual apparatus in the three delicate predacious Phycomycetes as consisting individually of oögonium, antheridium and oöspore, rather than of gametangia and zygospore, consideration was given more particularly to resemblances in general arrangement to the sexual apparatus figured by Arnaudow (**1**, *figs. 4, 5*) for *Zoophagus insidians* Somm., a predacious fungus of larger dimensions, whose position among the Oömycetes there has been little reason to question.

These resemblances and the possible taxonomic significance attaching to them are by no means yet to be dismissed. In any event, however, it is believed that the troublesome obscurity in structural detail like the attendant morphological ambiguity may in some measure be dispelled through an examination of several fungi of generally greater dimensions and more obvious distinctiveness, which have been found destructive to some of the larger species of terricolous *Amoebae*. Moreover, the fungi in question are well deserving of study in themselves, presenting remarkable novelties in vegetative development together with noteworthy peculiarities in sexual reproduction. And though the two variations in asexual reproduction would seem, when considered by themselves, rather commonplace both with reference to the shape of the conidia and to the arrangement in which the conidia are formed, when considered as pertaining to the Phycomycetes, they constitute departures as unexpected as they are difficult to homologize with the different types of asexual reproduction recognized in that class. Associated with the diversity in shape of thallus is a diversity in biological habit embracing endoparasitic, ectoparasitic and predacious relationships. Indeed, there are scarcely any distinctive features that are shared by all five species; yet each of the five shows

intimate similarities to one or more of the others, and the several correspondencies interlock in a manner leaving little doubt that one is dealing here with an assortment of naturally related forms.

ENDOCOCHLUS ASTEROIDES

Of the endoparasites, the one most frequently encountered on agar plate cultures attacks an *Amoeba* (PLATE 1, A) having a fairly substantial pellicle and finely granular transparent protoplasmic contents. The larger animals when in a more or less rounded condition measure approximately 60 μ in diameter, and exhibit clearly a single prolate elliptical nucleus (PLATE 1, A, *n*) about 15 μ long and 10 μ wide which contains two darker concavo-convex structures lying close within its periphery, one at each of the poles. A search through the literature has uncovered no description of any *Amoeba* with a nucleus of exactly such structure; though from the frequency of its occurrence on agar plates, the animal would seem to be abundant in soil and in decaying vegetable materials, and therefore could hardly be supposed not to have been encountered previously by protozoölogists. Undoubtedly the animal comes within the scope of Penard's (**15**, *p. 83*) statement: "Il me paraît donc préférable—de considérer comme *A. terricola* toutes les amibes terrestres, revêtues d'une pellicule, et pourvues d'un noyan unique et toujours ellipsoïdal." It will accordingly be designated here by Greeff's binomial, together, when necessary for more specific reference, with a numeral: *Amoeba terricola* I.

Contact between host and parasite is established as the animal moves along on the surface of the substratum on which conidia of the fungus are distributed; the distribution showing usually a noticeably linear arrangement wherein the positions of the aerial sporiferous hyphae, now evacuated and disintegrating, remain recognizable. The conidia thus encountered adhere to the pellicle of the host with sufficient firmness to withstand the jostling incident to continuing locomotion. Germination follows, a somewhat bulbous body being first produced, which apparently functions as an appressorium. From this bulbous part is thrust forth a tube of lesser diameter that perforates the pellicle and extends a distance of about 10 μ into the protoplasmic interior of the animal (PLATE 1, A, *a*). The tip of this tube now swells up into a globular

body which increases in size with the gradual transfer to it of all the material in the conidium (PLATE 1, A, *b*). When the transfer is accomplished, the globular body soon becomes disarticulated from the germ tube as the result of slight strains incident to the movements of the animal. The evacuated germ tube is then usually promptly expelled by the animal, but the globular body distinguished by the presence of a noticeable apiculum where it was earlier attached is retained within (PLATE 1, A, *c*, *c*) to be moved and jostled about passively in the protoplasmic streams together with the nucleus and often also with other inclusions like ingested protozoan cysts, testaceous rhizopods, and miscellaneous fungous spores in various stages of digestion. Sometimes the conidium, instead of remaining external to the *Amoeba*, is ingested either previous to germination or after development has begun, so that structures like that represented in plate 1, A, *a* may be observed being carried about in the protoplasmic streams. Even in these cases the evacuated membrane of conidium and germ tube apparently becomes separated from the globular body and is expelled, since instances of such a membrane remaining attached to thalli of larger sizes have never been seen in material of this species.

The number of infections to an animal depends obviously on the quantity of conidia encountered, which in turn stands in direct relation to the abundance of matured conidiophores. Early in the course of an epizoötic within a petri dish culture, an infected animal usually contains a single parasitic thallus, cases in which two or three thalli are visible occurring only now and then. As the conidia when produced are spaced at intervals approximately equal to the diameter of the *Amoeba*, it is apparent that a single infection would ordinarily result from the passage of an animal over a matured conidiophore at approximately a right angle, while two or three infections might well eventuate from passage at an oblique angle. Later when the epizoötic is well under way and conidiiferous hyphae span restricted areas here and there in an open arachnoid pattern, larger numbers of infections become more usual, so that animals carrying as many as a dozen thalli may be found. When such larger numbers of parasitic thalli are present, they may often be divided according to size into two or three categories, suggesting as in plate 1, A that the infections occurred in

several successive installments corresponding to successive passages of the animal over terrain strewn with conidia.

Vegetative growth of the young globular thallus takes place through widening and elongation at the distal end, at first apparently extending the original axis in a straight line (PLATE 1, A, *d*, *e*). When a length of approximately 10 μ has been attained, a rather abrupt curvature in direction intervenes, resulting first in an obese U-shaped structure (PLATE 1, A, *f*, *g*, *h*, *i*). With continued elongation at a more moderate, rather uniform degree of curvature and at a tolerably uniform diameter, modified sometimes by dichotomous branching at one or two points, a helicoid structure of striking appearance is brought into being (PLATE 1, B, C; PLATE 2, A, B). The ultimate size of this structure is conditioned naturally by the size of the animal as well as by the number of other thalli competing with it for the nourishment which the protoplasm of the host affords.

For a considerable period after infection the *Amoeba* shows virtually no evidence of ill effects from the presence of the fungus. The animal shown in plate 1, A, for example, although burdened with four thalli of more than negligible bulk in addition to five smaller ones, was at the time it remained under observation still moving about with apparently undiminished briskness. As the protoplasmic materials become more and more depleted, locomotion becomes gradually slower, the infected *Amoeba* at the same time evidently contracting in volume; yet even in decidedly advanced stages of debilitation (PLATE 2, A) when the bulk of the host has been reduced by perhaps more than a half and the cumbersome mass of fungous thalli on occasion visibly interferes with the free movement of the pellicle, some locomotion may be observed. Finally, of course, movement ceases, and the remaining protoplasmic host materials are absorbed, leaving virtually nothing of the animal except the collapsing pellicle (PLATE 2, B). With exhaustion of its food supply the fungus necessarily terminates its vegetative growth, and devotes its substance to reproduction, either sexual or asexual, or both.

In asexual reproduction the vegetative thallus gives rise usually to a single delicate filament, which, after perforating the enveloping pellicle, forces its way through the overlying substratum to

emerge in the air (PLATE 1, B, C). Aerial growth is continued until the protoplasmic contents of the fungous body have been used up, a length of 1.5 to 2 mm. being usually attained with fairly well developed specimens, while the largest thalli may give rise to aerial filaments twice as long. Sometimes a filament may give off one or more branches, usually near the point of egress from the substratum (PLATE 2, C). Branching however is never abundant, and in any case entails no increase in total length of filament produced.

Once the delicate aerial filament has attained its definitive length septa make their appearance in it at intervals usually of about 50 or 60 μ. Thereupon, mostly in positions approximately median between these septa, protrusions bud forth laterally (PLATE 2, G, *a*) and increase in size as the contents of the parent hyphal segments migrate into them (PLATE 2, H; G, *b*; I). The progressive evacuation of the segments is accompanied by the laying down of successive septa in the same manner as in aging mycelium of many species of *Pythium* and *Phytophthora*. When, therefore, the aerial filament becomes entirely evacuated it reveals cross-walls at intervals of from 5 to 30 μ, and bears at distances of 30 to 80 μ the individual conidia into which the protuberances have in the meantime developed (PLATE 1, C). The completed conidium is set off by a basal septum in a plane parallel with and usually tangent to the parent filament (PLATE 2, J, *b, c*), though in many instances the septum may be inserted a short distance from the axial hypha, so that the spore instead of always being accurately sessile is sometimes borne on a perceptible spur (PLATE 2, J, *a, d, e, f*). During maturation the protoplasm is withdrawn from the attenuated tip of the conidium, and the evacuated part, after being set off by a wall, persists as a small apical appendage (PLATE 2, J, *a–f*). Exclusive of this appendage the spore consists of a single fusiform colorless cell, surrounded by a thin smooth wall and filled with finely granular protoplasm. Under low or medium magnification, in undisturbed material, the slightly bristling and markedly linear arrangement of the conidia on the empty parent filaments, now matted down or loosely draped on the substratum, or often suspended like gossamer threads between projecting bits of solid material, provides a distinctive view. Such a view, however, is not

frequently afforded when cultures are infested with energetic creatures like mites, springtails, nematodes or small earthworms, for not only do the conidia become very readily detached from the parent filaments, but the parent filaments themselves become readily broken up even while the conidia are still in course of development.

Sexual reproduction of the fungus is initiated by the proliferation of hyphal outgrowths about twice as wide as those concerned with asexual reproduction. The outgrowths perforate the loosely enveloping host pellicle, and after growing out into the substratum a variable but usually very short distance, make apical or approximately apical contact with one another in pairs. Each pair of hyphae fuse at the place of contact, after which a spherical body develops, sometimes from the junction (PLATE 2, C, *d*), but much more frequently on a short extension arising from the junction or prolonging one of the hyphae (PLATE 1, B; PLATE 2, C, *a, b, c, e, g*; F). This body continues to increase in size with the movement into it of protoplasm from below until its definitive dimensions are attained, when it becomes delimited usually by a single basal septum (PLATE 2, C). The protoplast within the spherical cell thus formed then undergoes slow reorganization, developing a rather thick wall of its own (PLATE 2, D, *a–d*), which in time becomes contracted so as to present a stellate outer profile with six, seven or eight apices (PLATE 2, E, *a–h*). Between these apices the originally spherical enveloping membrane usually collapses slightly, resulting in relaxed, flat or even depressed facets. The living material occupying the spherical locule within the colorless or slightly yellowish stellate endogenous structure, shows at maturity a parietal layer of rather uniformly coarse granules surrounding a homogeneous central vacuole.

Although the structural relationships of the sexual apparatus are thus tolerably evident, some difficulties of interpretation remain. The very obvious, pronounced separation of the stellate structure from the membrane within which it is formed, is highly suggestive of the separation of oögonial wall and oöspore usual in such monosporous genera as *Pythium* and *Aphanomyces*. In a general way, too, the organization of the protoplasm would seem to conform to the organization usual in oöspores, though the stellate sculpturing injects optical difficulties that make it impossible to determine def-

initely whether or not a refringent body, the "helle Fleck" of de Bary, is present in the parietal granular layer. On the other hand, the relationships of the sexual hyphae to one another and to their joint product are certainly not those ordinarily obtaining between oögonium, antheridium and oöspore, but correspond essentially to relationships known among some Zygomycetes, such as those figured, for example, by Thaxter for his *Empusa echinospora* (**16**, *pl. 19, fig. 298–302*) and by Van Tieghem for *Syncephalis Cornu* Van Tiegh. & Le Monn. (**18**, *fig. 88–93*). From the account of the latter author, it would appear besides that *S. Cornu* presents similarity to the *Amoeba* parasite also in the development of a spiny spore within the spherical cell arising directly from the fusion of sexual elements, and in the presence of a large "oil drop" in the mature sexual spore. As in accordance with the very plausible "théorie dualiste" referred to by Lendner (**12**) the sexual spore proper of the Zygomycetes, no less than that of the Oömycetes, is essentially endogenous in origin, the development of the sexual apparatus in the present fungus would seem to require interpretation of the spherical cell as a zygosporangium, and of the stellate structure as a zygospore.

Not infrequently while the zygosporangium is still in course of enlargement, a septum appears in one of the sexual hyphae somewhere between its origin and its juncture with the other hypha. It seems at least questionable whether any special significance can be read into this septation, which in its inconstancy invites comparison with the occasional septation occurring in conjugating hyphal bodies of species of *Empusa*. There is little reason for believing that a septum in one of the sexual hyphae is different in function from a septum in the vegetative thallus itself, where a partition would seem to serve a purpose no more abstruse than the separation of a relatively large mass of protoplasm into portions destined for the production of separate reproductive units. In any case cross-walls are more wont to appear in the larger thalli that participate in the production of several zygosporangia besides giving rise, perhaps, to a conidiiferous filament, than in thalli so small that they become exhausted by participation in the development of a single zygosporangium or by proliferation of a single conidiiferous hypha.

To the extent to which the individual thalli can be distinguished from one another in the more or less intricate tangle into which they are usually compacted through the movements of their animal host, two conjugating hyphae seem regularly to originate from different thalli. Conjugation between filaments from thalli in separate *Amoebae* has never been observed, but presumably might well occur if two infected animals should die about at the same time and in close proximity to one another. Apart from the exigencies of a behavior strongly suggestive of heterothallism, the type of reproduction, whether sexual or asexual, is influenced appreciably by proximity to the air. When an infected *Amoeba* containing a number of thalli succumbs on or near the surface of the substratum, a more abundant development of conidia relative to zygospores ordinarily ensues than when the animal dies at a greater depth in the substratum. In the end, whatever the type of reproduction, the evacuated thallus membranes together with the pellicle of the host shrink into an inconspicuous wrinkled mass in which the separate constituents are indistinguishably confused (PLATE 2, D, E).

The parasite is apparently not eligible for inclusion in any genus of the Zygomycetes hitherto described. It would seem to represent the type of a new genus for which a name having reference to its curious spiral endozoic vegetative habit may be appropriate.

Endocochlus gen. nov.

Hyphae nutritae intra corpus amoebarum viventium evolutae, primo continuae, breviusculae, latae, simplices vel parum dichotomae, in spiras convolutae, animali emortuo extus hyphas conidiferas et hyphas zygosporiferas emittentes. Conidia aeria, hyalina, fusoidea vel ellipsoidea, hinc inde ex hyphis aeriis repentibus tenuibus arachnoideis oriunda assurgentia. Zygosporangia globosa intra materiam animal emortuum ambiens ex apice hypharum duarum similium conjugantium evoluta.

Vegetative hyphae endozoic, stout, simple or sparingly branched, when well developed often rather regularly and compactly convoluted, at first non-septate, after death of animal giving rise to conidiiferous and zygophoric hyphae. Conidia aerial, hyaline, fusoid or elliptical, borne singly at intervals on long aerial hyphae. Zygosporangia globose, produced in substratum outside of animal host at junction of two similar conjugating branches or on a short hyphal extension from such junction.

Endocochlus asteroides sp. nov.

Hyphae nutritae 4.5–8 μ diam., semel vel bis spiraliter convolutae. Conidia fusoidea, 11–19 × 3.2–4 μ, in apice appendicula 1.5–5 μ longa praedita, ex hyphis arachnoideis 1.2–1.4 μ crassis 1–4 mm. longis ad intervalla 30–80 μ longa enata. Zygosporae hyalinae vel luteolae, echinatae, intra zygosporangium spheroideum 11–14 μ diam. formatae, loculo 5–7.5 μ diam. Hyphae zygosporiferae 2.3–4 μ crassae, 30–65 μ longae.

Hab. in *Amoeba terricola* Greeff (in senso lato), Washington, D. C.

Vegetative hyphae 4.5 to 8 μ in diameter, simple or often when well developed sparingly branched dichotomously and compactly convoluted in 1 to 2 turns. Conidia spindle-shaped, measuring 11 to 19 μ (mostly 12 to 16 μ) in length by 3.2 to 4 μ (average about 3.6 μ) in diameter, exclusive of an empty apical appendage 1.5 to 5 μ (mostly 1.5 to 3 μ) long and about 1 μ wide at base; produced more or less erect and sessile or nearly sessile at intervals of 30 to 80 μ (mostly 50 to 60 μ) on aerial hyphae 1 to 4 mm. long and 1.2 to 1.4 μ wide. Zygospore colorless or slightly yellowish; at maturity broadly echinulate, the rather thick wall provided with about 20 protuberances of which 6 to 8 are visible in the stellate profile; containing a locule 5 to 7.5 μ in diameter; produced within a spherical zygosporangium 11 to 14 μ in diameter which arises mostly from a short hyphal extension from the junction of zygophoric hyphae that measure usually 30 to 65 μ in length and 2.3 to 4 μ in width.

Destructive to *Amoeba terricola* (in the broad sense more particularly of Penard) in cultures prepared from decaying roots of various herbaceous plants collected near Washington, D. C.

COCHLONEMA VERRUCOSUM

In a single agar plate culture a species of *Amoeba*, somewhat smaller than *A. terricola* I, was found parasitized by a fungus having much the vegetative habit of *Endocochlus asteroides*, but differing from it especially in asexual reproduction. When in a moderately rounded condition the larger individuals of the species of *Amoeba* attacked measured between 50 and 60 μ in diameter. This dimension, considered together with the slightly elliptical or nearly spherical shape of the nucleus (PLATE 3, E), the finely granular transparent character of the protoplasm and the delicate yet firm pellicle, permitted fairly plausible identification of the animal as *A. sphaeronucleolus* Greeff.

At the time the epizoötic was discovered it evidently had already progressed well towards its end, as all the individuals of the susceptible species still alive in the culture bore within themselves thalli of the parasite, varying in number from one to three, at different stages of development (PLATE 3, D, E). Consequently the entrance of the fungus into the animal could not be directly observed, a circumstance of lesser moment, however, as the mode of ingress could here be inferred even from material in advanced stages of development. For very generally to each of the endozoic thalli was still attached a small fusoid body with minutely verrucose membrane, readily recognizable as the conidium from which growth had proceeded (PLATE 3, C, D, E). Manifestly the conidium, after having been ingested by the hapless animal, had germinated laterally, the germ tube immediately widening out and directing its growth spirally, so as to yield a convolute thallus slightly less stout but otherwise resembling that of *Endocochlus asteroides*.

Asexual reproduction of the fungus is initiated when the contents of the animal have been reduced to such an extent that locomotion has virtually ceased (PLATE 3, D), though nucleus, contractile vacuole and remnants of cytoplasm usually continue for some time to present a fairly normal appearance (PLATE 3, E). Mostly from a position near the proximal end of the thallus is put forth a delicate hypha which perforates the pellicle and, if the animal is at all submerged, makes its way to the surface of the substratum. Near or on this surface, if it has not already become branched, the filament undergoes some ramification (PLATE 3, C, E; PLATE 4, A). The resulting branches after spreading in divergent directions for variable distances, grow up into the air, where each gives rise to a more or less erect aerial chain of usually 30 to 40 fusoid verrucose conidia (PLATE 4, A).

The chains of conidia show the general features associated with the genus *Fusidium* in the Hyphomycetes. In spite of the considerable attention devoted to the details of spore formation in this species as well as in the other three catenate forms to be described, it has not been possible, owing to difficulties attending observation, to determine this phase of developmental morphology with as much certainty as might be desired. The structures concerned are too small to be studied successfully in their normal

aerial state under a dry objective. On the other hand when developing and hence still immature sporogenous filaments are mounted in water, their appearance suggests so strongly a prompt intervention of degenerative change that the normal course of events remains somewhat a matter of inference. However, judging from the more satisfactory microscopic preparations, it would seem that the spore chains have origin in continuous aerial filaments which from an early stage, if, indeed, not from the very beginning, are characterized by *Leptomitus*-like constrictions spaced at equal intervals (PLATE 3, E; PLATE 4, A, *b, c*). These regularly constricted filaments exhibit minute verrucose sculpturing that does not extend proximally into the sterile parts of the supporting hyphae. Apparently some slight increase in size takes place before the distended parts become separated from one another through withdrawal of protoplasm from the constricted isthmi and formation of a delimiting partition at either end of the individual swellings (PLATE 4, A, *a, d*). In a mature condition the chains are very easily broken up, so that the conidia soon come to lie separately on the surface of the substratum (PLATE 3, F), awaiting the passage of a susceptible animal, and a repetition of the developmental cycle just outlined.

Sexual reproduction of the fungus was observed in connection with but a single animal, and in this was represented by only two unions (PLATE 3, G). Unfortunately, moreover, the membranes of the empty thalli and their hyphal outgrowths were already so badly collapsed that none of the zygophores could with certainty be followed backward any considerable distance from junction to origin. The junction, which in both cases was revealed plainly, appeared similar to that in *Endocochlus asteroides*, and as usually in the latter fungus bore the zygosporangium on a short prolongation. The zygosporangium here, however, was much more distinctly yellowish in coloration, and instead of being smooth, bore numerous prominent bullate protuberances. Within this sculptured primary fusion cell, to extend the contrast, was revealed in the one nearly mature specimen a zygospore with a smooth wall of moderate thickness.

In shape and sculpturing of conidium as well as in the persistence of that structure on the endozoic thallus, the fungus shows

obvious similarities to the form which Penard (**15**) found parasitizing *Amoeba alba* Greeff and discussed as *Saprolegnia* B. Although the Swiss zoölogist did not succeed in observing the production of the conidia described by him, very probably because he employed mostly cultures of aquatic rather than terrestrial character, their close correspondence to those of the present fungus gives much ground for believing that he may have been dealing with an intimately related catenulate form. It may be mentioned that a cochleate endoparasite with catenulate conidia of approximately the large dimensions given by Penard occurred some years ago on an unidentified *Amoeba* in one of my agar plate cultures, though because of unfamiliarity with the type of fungus in question, which was then mistakenly judged to be referable to *Fusidium,* the scanty material was dissipated before more accurate records had been made of morphological details. However, the method of reproduction by zoöspores that was attributed, even if only with partial assurance, to *Saprolegnia* B, has never been observed either in the inadequately studied species or in any of the catenulate forms dealt with herein; nor would the swollen rather irregularly branching mycelium figured by Penard appear to conform any too well to the regularly involute type of endozoic thallus.

Since the mode of asexual reproduction represented here differs conspicuously from that set forth as characteristic of *Endocochlus,* a separate genus is proposed:

Cochlonema gen. nov.

Hyphae nutritae intra corpus amoebarum viventium evolutae, primo continuae, breviusculae, latae, simplices vel parum ramosae, in spiras convolutae, animali emortuo vel moriente extus hyphas conidiferas et hyphas zygosporiferas emittentes. Conidia aeria, hyalina, fusoidea vel elongata, in catenulas longiusculas simplices plus minusve erectas digesta. Zygosporangia globosa, intra materiam animal emortuum ambiens ex apice hypharum duarum similium conjugantium evoluta.

Vegetative hyphae endozoic, stout, simple or sparingly branched, when well developed often rather regularly and compactly convoluted, at first continuous, with decline or on death of host animal giving rise to delicate conidiiferous and usually somewhat stouter zygophoric filaments that pass through the host envelope. Conidia aerial, hyaline, fusoid or elongated, produced in long

chains from more or less erect aerial hyphae. Zygosporangium globose, produced on a short prolongation from the apical junction, or directly from the junction of two similar filaments, in the material surrounding or underlying the animal.

Cochlonema verrucosum sp. nov.

Hyphae nutritae 4.5–7 μ diam., semel vel bis spiraliter convolutae. Conidia verruculosa, fusoidea, 6–9 $\times$ 1.4–2 μ, ex hyphis saepius 1.2–1.5 μ crassis enata, in quaque catenula tricena usque quadragena. Zygosporangia flavida, globosa, circa 12 μ diam., 20–30 verrucis ornata; verrucae 1.5–2 μ altae, basi 2.5–3 μ diam.; zygosporae globosae. Hyphae zygosporiferae breviusculae, 2–3 μ crassae.

Hab. in *Amoeba sphaeronucleolo*, Washington, D. C.

Vegetative hyphae usually 4.5 to 7 μ in diameter, simple or often when well developed sparingly branched and rather compactly convoluted in 1 to 2 turns. Conidia minutely verrucose, fusoid, measuring 6 to 9 μ in length by 1.4 to 2 μ in diameter, produced in chains usually of 30 or 40, on hyphae mostly 1.2 to 1.5 μ wide. Zygosporangia yellowish, globose, measuring about 12 μ in diameter exclusive of the warty protuberances 20 to 30 in number that measure often 2.5 to 3 μ in basal diameter and 1.5 to 2 μ in height; zygospores smooth, globose; zygophoric hyphae rather short, mostly 2 to 3 μ wide.

Destructive to *Amoeba sphaeronucleolus* in a laboratory culture, Washington, D. C.

COCHLONEMA DOLICHOSPORUM

A fungus very similar to the one just described was found in small quantity in another agar plate culture destroying *Amoebae* having approximately the same dimensions as *Amoeba sphaeronucleolus*. Animals in stages of infection early enough to reveal their nuclear condition were not observed, however, so that the identity of the host remains for the time being uncertain. As in the case of *Cochlonema verrucosum* the conidium here is evidently ingested bodily, since it likewise is regularly to be seen attached to the endozoic thallus, though attached somewhat less closely by a delicate germ tube of appreciable length (PLATE 3, A; PLATE 4, B). Asexual reproduction results here also in chains of aerial conidia (PLATE 4, B, *a*, *b*, *c*), the individual conidium being, however, two or three times as long, somewhat more prominently sculptured, and often when fully mature evacuated of protoplasm

in the distal narrower part, which then nevertheless persists as an empty appendage (PLATE 3, B). Accordingly, whereas the conidium when newly delimited is ordinarily slightly obclavate in shape, after evacuation of the appendage the cell left filled with protoplasm is more nearly symmetrical, that is, roughly elongated fusoid in shape. Sexual reproduction has not been observed.

Cochlonema dolichosporum sp. nov.

Hyphae nutritae circa 6 μ diam., semel vel bis spiraliter convolutae. Conidia verrucosa, 15–25 $\times$ 1.2–2 μ, ex hyphis 1–1.5 crassis enata, in quaque catenula quina usque vicena, primo saepius nonnihil obclavata, maturitate apice saepius vacuo, tum cellula viventi plus minusve fusoidea vel cylindracea. Zygosporae ignotae.

Hab. in *Amoeba* sp., Washington, D. C.

Vegetative hyphae approximately 6 μ in diameter, convolved into a spiral of 1 to 2 turns. Conidia verrucose, measuring 15 to 25 μ in length and 1.2 to 2 μ in width, borne in chains of 5 to 20 each on hyphae 1 to 1.5 μ wide, at first somewhat broader in proximal than in distal portion, later often becoming evacuated in the narrower apical part, which then persists as an appendage up to 7 μ long on the cylindrical or somewhat fusoid living cell. Zygospores unknown.

Destructive to *Amoeba* sp. in a laboratory culture, Washington, D. C.

BDELLOSPORA HELICOIDES

A fungus strongly resembling the two species of *Cochlonema* in its asexual reproduction was observed in a number of agar plate cultures, usually in quantity, habitually parasitizing a species of *Amoeba* the larger individuals of which when fairly well rounded up measured from 60 to 90 μ in diameter. The cytoplasm of the animal was in general finely granular, colorless and decidedly transparent; its pellicle firm, perhaps rather thicker than in most terrestrial forms, and therefore inclined to be cast into fewer but more pronounced folds. The single nucleus (PLATE 5, B, *n*) having the shape of a prolate ellipsoid of revolution showed at the poles accumulations of darkish irregular lumps that thinned out to a single layer in the equatorial periphery. The structural features of the animal thus correspond tolerably well to those that Penard (**15**) ascribed to his *A. terricola* var. *papyracea*. Yet no

close approximation to the considerably larger dimensions cited by Penard has ever been observed in my material, so that a presumption of identity would seem at least doubtful. The animal, of course, readily conforms to Penard's broad concept of *A. terricola,* and may thus be conveniently referred to as *A. terricola* II, the appended numeral being intended to distinguish it from the habitual host of *Endocochlus asteroides,* as well as from the *Amoeba* attacked by the fungus to be described as *Zoopage phanera.*

Infection is initiated in much the same way as was described in the account of *Endocochlus asteroides.* Conidia of the parasite strewn about on the surface of the substratum (PLATE 5, A) make contact with the *Amoeba* as it passes over them and remains adhering to the pellicle in spite of subsequent locomotion of the animal both on and through the substratum. Each adhering spore soon puts forth a delicate germ tube which perforates the pellicle and continues in its course into the host for a distance often about equal to the length of the conidium (PLATE 5, B, *a*). From this stage on, analogy with *E. asteroides* ceases. The tip of the infective germ tube instead of developing a globular body into which the conidial contents are received, becomes dichotomously branched (PLATE 5, B, *b–f*). Each of the divergent limbs soon branches in its turn, and in a plane at a right angle to that of the original dichotomy (PLATE 5, B, *g*). A third bifurcation follows the second (PLATE 5, B, *h*), and in some cases a fourth bifurcation takes place (PLATE 5, B, *i*), so that eventually a compactly branching apparatus with 8 to 16 terminal elements is brought into being. This apparatus has all the appearance of a haustorium and, indeed, manifestly is one; for coincident with the first bifurcation the spore outside of the animal begins to show perceptible swelling (PLATE 5, B, *a–f*). This swelling continues until the conidium has expanded into an obese ellipsoidal body whose mode of origin is later often betrayed only in two minute protruding polar apiculi representing the apparently inelastic spore extremities (PLATE 5, B, *i*).

While in the early stages of an epizoötic individual *Amoebae* are often found with but a single infection, plural infections become the rule subsequently, when with an increasingly abundant

and thoroughgoing distribution of conidia the encounters between host and parasite become more and more frequent. Accordingly in the more advanced phases of an epizoötic, animals beset with ten or a dozen separate plants in various stages of development are not of infrequent occurrence (PLATE 5, B). Locomotion although impeded physically by the presence of the larger swollen spore bodies, usually continues for some time after the progressive exhaustion of protoplasmic contents has entailed a readily noticeable contraction of the animal's bulk. Its cessation, in fact, ordinarily is brought about not primarily by exhaustion of the host as in the case of *Amoebae* infected with *Endocochlus asteroides*, but through virtual anchoring of the host to or in the substratum that takes place with the proliferation of hyphal outgrowths from spore bodies preparatory to reproduction. The anchored animal continues to furnish food materials to the parasite during a protracted period of reproductive development, so that conidial chains (PLATE 6) or sexual apparatus (PLATE 5, C) may be displayed in abundance even while the host animal is still alive. The parasitized *Amoeba* in the last stages preceding death is rounded up into an almost spherical shape, its pellicle showing few irregularities except for the depressions where the spore bodies are attached (PLATE 5, C; PLATE 6). Even with the animal reduced to perhaps a third of its original bulk, the granular material now remaining suffices only to provide a thin parietal layer surrounding an immense central vacuole. Often protruding conspicuously from the parietal cytoplasmic layer into the central vacuole is a vesicle-like vacuole, evidently to be interpreted as the contracting vacuole, now greatly enlarged apparently owing to its incapacity to discharge through the thickened pellicle.

As has been intimated asexual reproduction of the ectoparasite follows the same general course as in *Cochlonema verrucosum* and *C. dolichosporum*. Like the convolute endozoic thalli of these fungi, the swollen spore bodies here put forth a single hypha, or less often several hyphae. Each filament, after traversing the substratum for a variable distance depending in some measure at least on the depth at which the host animal was halted, reaches the surface where immediately or after some prostrate growth its further extension becomes aerial and more or less erect. One or

several branches may be given off in either the intramatrical (PLATE 6, C), the superficial (PLATE 6, B), or the aerial (PLATE 6, A–D) part. In any case, on each of the resulting aerial hyphae is formed terminally a chain (PLATE 6, A, *a, c*; B, *b, d*; C, *a, b*; D) of irregularly fusiform conidia, between a half-dozen and a score in number. With an adequate supply of nourishment development of a second chain of spores from a branch having origin just below the base of the first chain (PLATE 6, A, *b, d*; B, *a, c*), and even of a third chain from a second branch, may ensue. On maturity the chains are broken up on relatively slight disturbance, as, for example, on being brushed by passing nematodes; so that the conidia are soon strewn about on the substratum, ready to infect any susceptible *Amoeba* that may happen to come along.

The external position of the spore bodies on the animal, and their globose shape that makes it easy to distinguish them from one another even under crowded conditions, provide in this species circumstances more favorable for determining the relationships involved in sexual reproduction than obtain in any of the related convoluted endozoic parasites. In spite of a frequently somewhat intricate arrangement here of the zygophoric hyphae themselves, two conjugating filaments can always be traced back to separate spore bodies, never to the same spore body (PLATE 5, C). These filaments whether representing direct outgrowths of their respective spore bodies (PLATE 5, C, *d*), or primary (PLATE 5, C, *c*) or even secondary branches of such outgrowths (PLATE 5, C, *a, b*), usually engage one another very close to the swollen structures, if not in immediate proximity to them. After establishing apical contact they continue to elongate, twisting about one another in conspicuously regular close helical turns. Each of the hyphae makes from two to four turns, the total number of windings in the intertwined helices thus varying usually between four and eight. No discrimination with respect to direction of rotation, whether dextrorse or sinistrorse, is evident.

The intervolved hyphae now fuse apically or approximately apically. On the end of a short cylindrical part continuing one of the hyphae or arising more symmetrically from the junction, a globose body buds out and increases in size to form a zygosporangium (PLATE 5, C). By this time a septum is present in each of

the zygophores usually at a point about median in the intervolved part. The distal halves of the zygophoric filaments delimited proximally by these septa constitute elements that would seem to correspond more plausibly to the gametangia of the more familiar genera in the Zygomycetes than any structural constituents of the sexual apparatus produced by any of the other fungi herein described, or by any of the minute predacious forms figured earlier (**9**, *figs. 3–5*). After the terminal zygophoric parts have contributed their contents to the zygosporangium, the latter is partitioned off from its supporting element by a basal septum. In the meantime the originally smooth zygosporangium has become sculptured through the putting forth of noticeably thick-walled wart-like or bullate protuberances. Internal development now ensues with the result that finally the sculptured wall comes to envelop rather closely a zygospore proper, which at maturity has a smooth wall of moderate thickness, and within this wall a parietal layer of uniformly and rather coarsely granular material surrounding an apparently homogeneous, relative large reserve globule (PLATE 5, D–H, I, *a–h*). A clustered arrangement of these distinctly yellowish sexual structures in the substratum about a wrinkled residual mass in which the shrunken pellicle of the host and the collapsed membranes of the parasite are mostly unrecognizably confused, remains to mark the place where an animal came to its end (PLATE 5, I). Indeed, just as in the case of *Endocochlus asteroides,* the presence of such clustered arrangements often constitutes the only testimony to a former abundance of the susceptible *Amoeba* species in cultures from which it has been exterminated.

The close resemblance of the zygosporangium and zygospore to those of *Cochlonema verrucosum* suggests the possibility that the remarkable intervolution of zygophores in the present fungus, so reminiscent of the relationships of *Syncephalis nodosa* van Tiegh. as illustrated in the beautiful figures of Bainier (**2, 3**) and of Thaxter (**17**), may constitute a feature having significance only as a character pertaining to the species. A consideration of sexual as of asexual reproduction, therefore, reveals no decisive reason for not assigning the fungus to *Cochlonema.* However, the remarkable epizoic habit with its striking analogy to the habit

prevalent among the Rhizidiaceae in the Chytridiales, offers so direct an antithesis to that exemplified in *Cochlonema* and *Endocochlus* that assignment to either of these genera would seem inappropriate. A separate genus is accordingly proposed under a name which is intended to bring into relief the leech-like behavior, so to speak, of the conidium.

Bdellospora gen. nov.

Conidia aeria, hyalina, fusoidea vel elongata, in catenulas longiusculas simplices plus minusve erectas digesta; ad pelliculam animalium adhaerentia, hypha germinationis pelliculam perforantia, haustorium ramosum intus evolventia, tum magnopere tumescentia, mox hyphas conidiferas et hyphas zygosporiferas emittentia. Zygosporangia globosa intra materiam animal emortuum ambiens ex apice hypharum duarum similium conjugantium evoluta.

Conidia aerial, hyaline, fusoid or elongate, arising in frequently long chains from more or less erect aerial hyphae; adhering to animals and after individually perforating the pellicle or integument by means of a germ tube that develops into a haustorium inside, swelling into large globose-ellipsoidal bodies from which conidiiferous and zygophoric hyphae grow out. Zygosporangia globose, developed in the material surrounding or underlying the dying animal from the junction of two similar conjugating filaments.

Bdellospora helicoides sp. nov.

Conidia fusoidea, nonnihil angulata, 6–16 × 2–3 μ, ex hyphis 1.3–2 μ crassis enata, in quaque catenula quina usque vicena; post amplificationem globoso-ellipsoidea, usque 15 μ diam., 20 μ longa, haustorio pedicellato usque ter vel quater breviter bifurcato. Hyphae zygosporiferae inter se quater vel octies spiraliter circumplicantes, utraque ex conidio turgido alio enata, basi saepius 2–2.5 μ crassae, sursum 3–4.5 μ crassae, 30–75 μ longae, septo in partes duas fere subaequaliter divisae. Zygosporangia 8–13 μ diam., flavida, 10–30 verrucis 1–1.5 μ altis, 1.5–3 μ diam. ornata. Zygosporae globosae.

Hab. in *Amoeba terricola* (in senso lato), Washington, D. C.

Conidia fusoid, measuring 6 to 16 μ (average 10 μ) in length by 2 to 3 μ (average 2.7 μ) in width, somewhat angular in outline, produced in chains usually of 5 to 20 on aerial hyphae mostly 1.3 to 2 μ wide; after vegetative enlargement globose-ellipsoidal, measuring up to 15 μ or more in transverse diameter, and up to 20 μ or more in length, each conidial body being provided with a haustorium consisting of a germ pedicel 3 to 7 μ long and up to 1.5 μ wide together with short elements up to 2.5 μ wide

in a terminal closely dichotomous branching system. Zygophoric hyphae 4 to 8 times intervoluted, each of a conjugating pair arising from a separate swollen conidium, measuring 2 to 2.5 μ in diameter at the base but widening toward the apex to a diameter of 3 to 4.5 μ, mostly 30 to 75 μ long, and regularly divided by a transverse septum into 2 nearly equal parts. Zygosporangia mostly 8 to 13 μ in diameter, distinctly yellowish, ornamented with 10 to 30 (mostly about 25) wartlike protuberances measuring 1 to 1.5 μ in height and 1.5 to 3 μ in basal diameter. Zygospores globose, smooth.

Parasitic on *Amoeba terricola* (in the broad sense especially of Penard) in laboratory cultures made from decaying rootlets collected near Washington, D. C.

ZOOPAGE PHANERA

More readily visible in its results than any of the parasitic relationships herein discussed is a predacious relationship involving as prey a large and relatively opaque species of *Amoeba* that often becomes very abundant in aging plate cultures. Though the species is somewhat larger even than the one attacked by *Bdellospora helicoides*, measuring between 35 and 110 μ in diameter when drawn up into a more or less rounded shape, its pellicle is so delicate as to appear under high magnification as a single-contoured membrane. The cytoplasm of the animal consists of granular material in much larger proportion than in most terricolous forms, and accordingly presents an appearance closely simulating that of the plasmodia of various myxomycetes often present in the same cultures. In its rather dark and almost opaque cytoplasmic matrix the single nucleus is not easily to be found (PLATE 7, A, *d*), yet at opportune moments it can be discerned as a prolate elliptical body composed of a central hyaline part surrounded by a slightly darker external layer (PLATE 7, A, *n*). While the animal thus conforms to Penard's broad concept of *A. terricola* it has an appearance much different from the appearance of the habitual host of either *Endocochlus asteroides* or *B. helicoides*; and would seem, moreover, to be as completely immune from being parasitized by these fungi as their habitual hosts are immune from capture by the fungus preying upon it. In view of these differences, and of what would seem to be a somewhat indiscriminate

application of Greeff's binomial, requirements for more specific reference suggest again the use provisionally of an appended numeral in the designation *A. terricola* III.

The fungus destructive to this *Amoeba* is represented in its vegetative stage by a non-septate mycelium branching at moderate intervals both within and on the surface of the substratum, the branches being directed usually at wide angles to their parent filaments in a seemingly ill-defined haphazard manner (PLATE 7, A). Here and there the hyphae show noticeable though usually not pronounced variations in diameter. The commonplace morphology of the diffuse mycelium together with the absence of visible adhesive material is not at all suggestive of a predacious habit. When, however, an animal in the course of its customary wanderings makes contact with a mycelial filament, the contact proves unexpectedly persistent and tends to increase in extent, so that a certain measure of inwrapment often results, especially in cases where additional filaments nearby are also engaged (PLATE 7, A, *a*). From its applied surface the mycelium buds forth at intervals delicate processes that perforate the pellicle, penetrate a short distance into the body of the animal to give rise there individually to several swollen lobules in botryoid arrangement (PLATE 7, A, *a–d*). Though these endozoic processes are of rather small dimensions and ordinarily do not exceed a half-dozen in number, their intrusion is soon followed by obvious degenerative changes in the animal's cytoplasm. The *Amoeba* here concerned, unlike the forms parasitized by *Endocochlus asteroides* and *Bdellospora helicoides*, shows little endurance to internal attack, usually succumbing before the cellular contents have become greatly reduced (PLATE 7, A, *b, c*).

If the general course of the predacious relationship can be followed without any difficulty, the precise manner of capture is a matter of inference rather than of direct observation. The stalked processes with their botryoid terminations present, it is true, much the appearance of grappling organs, but whether they actually function as such seems somewhat unlikely, as the semi-liquid or perhaps softly gelatinous consistency of the protoplasm in which they are immersed can hardly be assumed to furnish anchorage firm enough to resist the ordinary locomotor pull of the animal.

Moreover the very production of the processes must have as an antecedent intimate contact for a more or less protracted period between parent hypha and pellicle. Since this contact is maintained in spite of the animal's restricted but appreciably lively movements in all directions, it may be presumed that adhesion is operative here even though visible deposits of sticky material cannot be distinguished. In mode of application a close similarity to the conidium of *Bdellospora helicoides*, which likewise adheres to its host without an adhesive substance being visible, thus becomes apparent. The similarity is extended in an unmistakable correspondence between the endozoic parts growing from the adhering structures, in accordance with which the delicate stalked processes are interpretable as haustoria rather than as capturing organs.

In whatever the apparatus of capture may consist, as to its efficacy at least there can be no question. When a soft agar medium has been used in making a culture, the *Amoebae* move about freely in or through as well as on the substratum, and are then caught largely here and there on the submerged mycelial hyphae without becoming concentrated anywhere so as to cause much of a display. With the employment of a harder agar medium, however, the animals are constrained to live, feed and move about exclusively on the surface of the culture; and naturally it is then only on the surface that they are captured. As a result scores and even hundreds of *Amoebae* in various stages of decline or disintegration then often accumulate on the restricted areas occupied by separate mycelial tracts of the fungus, becoming readily visible to the naked eye as a superficial deposit of greyish stippled aspect. At later stages the deposit usually is obscured somewhat under a whitish pulverulent efflorescence, which upon microscopic examination is revealed as consisting of a profuse tangle of conidial chains.

In addition to their origin in catenulate arrangement the conidia of the fungus show resemblances to those of *Cochlonema verrucosum* and *Bdellospora helicoides* in their generally fusiform shape as well as in the sculpturing of their enveloping membranes (PLATE 7, A, *f*, *g*; B, *a–c*). The spore chains are borne on usually rather short, erect, distally attenuated branches that arise without much

regularity from the superficial hyphae. Because the chains are usually long—lengths of from .5 mm. to 1 mm. being not infrequent—relative to their width especially at the constricted connections, they usually droop until the distal portions come in contact with the substratum. On maturation they are broken up into the separate conidia when disturbed even slightly as by the jostling of a passing nematode.

Sexual reproduction of the fungus takes place abundantly in the substratum underlying the conidial chains. Two branches, one often more or less contorted (PLATE 7, B, *d*, *e*; C–G), the other more nearly straightforward in course, arising from separate elements, whether mycelial filaments (PLATE 7, B, *d*, *e*; C–H) or germinating conidia (PLATE 7, N, O), encounter one another, fusion takes place, and at the junction a spherical body makes its appearance. This body increases steadily in size, being supplied with protoplasm from both branches, with each of which it communicates directly, never indirectly through a single common prolongation as in the sexual apparatus of the parasites previously described herein. During the earlier stages the branches remain continuous, but later, usually when the globular body is well along in development, a cross-wall appears in one or both of them at some distance from the body. On attaining definitive size the spherical body is delimited from each of the branches by an approximately tangential septum (PLATE 7, G, H). Within the smooth zygosporangium thus formed is developed the zygospore proper, a distinctly yellowish structure which at complete maturity reveals a handsomely sculptured, bullate wall, a parietal layer of uniformly coarsely granular material and a central reserve globule (PLATE 7, I–M). The zygosporangial membrane generally collapses somewhat between the bullate protuberances, though remaining recognizable usually as a separate envelope.

The predacious habit of the fungus entails such a marked difference in morphology of vegetative thallus from the genera *Cochlomena* and *Bdellospora* that the proposal of an additional genus, under a name constructed from two words meaning "animal" and "anything that fixes or fastens" respectively, appears justified.

Zoopage gen. nov.

Mycelium effusum; hyphis ad pelliculam animalium adhaerantibus, ramulis hanc perforantibus, haustoria intus evolventibus. Conidia aeria, hyalina, fusoidea vel elongata, in catenulas saepe longas plus minusve erectas digesta. Zygosporangia globosa, intra materiam ambientem vel subjacentem ex copulatione hypharum similium orta.

Mycelium effuse, the hyphae adhering to the pellicle or integument of an animal, perforating it and producing a haustorium inside. Conidia aerial, hyaline, fusoid to elongated, arising in more or less erect chains from aerial branches. Zygosporangia globose, produced in the substratum at the junction of two conjugating hyphae.

Zoopage phanera sp. nov.

Mycelium ramosum; hyphis hyalinis, 1.2–2.7 μ crassis; haustoriis pedicellatis, pedicello .5–1 μ crasso, 3–6 μ longo, 3–7 lobulos turgidos saepe 2–3 μ longos et crassos ferente. Conidia minute verrucosa, elongato-fusoidea, 25–60 μ, saepius 35–45 μ longa, 2.2–2.8 μ crassa, ex apice hypharum saepius brevium oriunda, in quaque catenula quina usque vicena quina. Zygosporangia 9–12 μ diam., primum levia, maturitate nonnihil collabentia. Zygosporae flavidae, 6.5–10 μ diam., loculo 5–7 μ diam., membrana .6–1.5 μ crassa 15–30 verrucis ornata.

Hab. in terra, *Amoebam terricolam* (in senso lato) capiens et consumens, Washington, D. C.

Mycelium branched; hyphae 1.2 to 2.7 μ wide; haustoria pedicellate, the pedicels, mostly .5 to 1 μ in width and 3 to 6 μ in length, bearing apically in botryoid arrangement from 3 to 7 lobulations measuring 1.5 to 6 μ, mostly 2 to 3 μ, in length and in thickness. Conidia elongated fusiform, minutely verrucose, measuring 25 to 60 μ, mostly 35 to 45 μ (average 40 μ) in length and 2.2 to 2.8 μ (average 2.4 μ) in width, produced in chains of 5 to 25 on distally attenuated, mostly short branches. Zygosporangium 9 to 12 μ in diameter, at first smooth, at maturity collapsing somewhat about the sculptured zygospore. Zygospore yellowish, 6.5 to 10 μ in diameter, with a locule 5 to 7 μ in diameter and a wall .6 to 1.5 μ thick; the wall provided with 15 to 30 verrucose protuberances, of which 6 to 8 are visible in profile on its outer sigillate contour.

Capturing and destroying *Amoeba terricola* (in the broad sense more particularly of Penard), Washington, D. C.

TAXONOMIC CONSIDERATIONS

The similarity of the intervolved zygospores of *Bdellospora helicoides,* even in the absence of bladder-like appendages, to the

spirally intertwined sexual branches of *Syncephalis nodosa* directs attention to the possibility of a homological relationship between the conidial chains produced in three of the newly erected genera with the rows of asexual spores characteristic of *Syncephalis, Piptocephalis* and *Syncephalastrum*. An important difference is, of course, at once apparent in the absence here of anything at all corresponding to the simple or branching, usually rather stout, erect hyphae whose remarkably varied capitate development must have facilitated the plausible interpretation of the ultimate cylindrical elements borne on them as constituting either linear sporangia or sporangial ramuscules, wherein the spores though formed in a row are nevertheless formed endogenously much as in species of *Mucor* or *Rhizopus*. It might be argued with some cogency, however, that endogenous formation of asexual spores could conceivably occur in the Zygomycetes unassociated with the easily recognizable correspondencies of external morphology through which homologies with the very familiar spherical type of sporangium have been brought into relief; that, in fine, an element approximately homologous to a linear sporangium or sporangial ramuscule might be formed, without the intervention of a conspicuously differentiated sporangiferous hypha, directly on a commonplace filament. The proof of endogenous spore production here would necessarily depend more nearly exclusively on optical evidence, like that adduced by Thaxter (**17**) in the case of *Syncephalastrum racemosum* Cohn, showing physical separateness of the spore wall from a sporangial wall or ramuscule wall enveloping it.

No such conclusive evidence bearing on sporulation in the four catenulate fungi described herein has come to light, though a few morphological details permit of possible alternative interpretations favorable to endogeny. Thus the development of spore chains from originally continuous filaments with regularly spaced constrictions might be taken to imply parallelism with the type of spore formation illustrated by Thaxter in several figures (**17**, *pl. 1, figs. 9–11*) of his *Syncephalis Wynneae*; or, on the other hand, it might be construed as representing merely a modification of the type of spore formation familiar in *Oospora lactis* Fres. Again the presence of sculpturing on the conidia of all four catenulate species, when the conidia of comparable non-catenulate *Amoeba-*

destroying fungi are uniformly smooth, might or might not be taken to indicate parallelism with *Syncephalis intermedia* Van Tiegh. and *Syncephalis nodosa*, where according to Van Tieghem (**18**) the wrinkled exterior is attributable to the persistence of the segment of the sporangial envelope surrounding the conidium proper.

In any case, concerning the intimate relationship of these non-catenulate *Amoeba*-destroying forms with the catenulate forms there assuredly can be no serious doubt. The strong similarity in vegetative habit between *Endocochlus asteroides* and *Cochlonema verrucosum*, supported by a general parallelism in make-up of sexual apparatus, provides adequate testimony to a close affinity between these two species. Nor are the two types of conidial production exemplified in these species entirely irreconcilable with one another. The segments resulting from the insertion of the first order of septa in the conidiiferous hyphae of *Endocochlus* can without much straining be homologized with the seriate swellings in the young spore chains of *Cochlonema*; the lateral conidia proliferated from them then becoming interpretable as the immediate exogenous products of structures equivalent to catenated conidia. The sessile conidia of three of the delicate Phycomycetes capturing rather small *Amoebae*, which were figured in my earlier note (**9**, *figs. 2, 4, 5*), appear certainly morphologically equivalent to the conidia of *Endocochlus*, even though they are produced from prostrate, predacious and hence primarily vegetative hyphae rather than from primarily reproductive aerial filaments. In the two (**9**, *figs. 4, E*; *5, E, F*) of the three forms for which sexual reproduction is known, this type of reproduction is closely similar to that found in *Zoopage*, the small originally smooth zygosporangium communicating separately with each of the outwardly almost undifferentiated zygophoric branches; and its wall later collapsing slightly over a sculptured zygospore. Precisely the same make-up of sexual apparatus is represented also in a fourth delicate predacious form whose small conidia are produced successively on a delicate erect sporophore (**9**, *fig. 3*). A simplification of this multiple proliferation of conidia appears in an *Amoeba*-capturing fungus yet to be described, which gives rise to an elongated obovoid conidium, measuring on an average about 15 μ in length

by 6.4 μ in diameter, at the tip of an erect conidiophore usually about 200 μ long and .8 μ wide. This form is of especial importance as it reveals such thoroughgoing parallelism with the much larger Phycomycete capturing nematodes by adhesion, which was figured in a brief summary (**8**, *fig. 8*), that a close natural relationship is sufficiently obvious.

Through the ramification of overlapping resemblances, therefore, the five fungi newly described are shown to be related rather intimately with forms differing greatly from them and from one another in the arrangement and dimensions of their conidial apparatus. It may be presumed that fungi of similar morphology have been encountered by investigators from time to time; yet, aside from some descriptions of filamentous growths associated especially with protozoans, hardly any mycological writings can be referred at all plausibly to the group under consideration. The *Saprolegnia* B of Penard seems almost certainly to belong here, though the production of zoöspores attributed to the fungus is little consonant with development in the Zygomycetes generally. The mycelium which Dangeard (**4**) described as attacking an *Amoeba* by means of a bifurcating haustorium, and to which in the absence of asexual and sexual reproductive structures he applied the binomial *Rhizoblepharis amoebina*, might be referred to the group under discussion with more plausibility if dichotomous branching of the haustorium did not also occur, and, indeed, in much greater measure, in the *Amoeba*-capturing fungus figured earlier (**9**, *fig. 1*), whose freely septate mycelium and septate, appendaged conidium rather clearly indicate mucedinaceous affinities. Possibly the filamentous outgrowths that on being found attached rather consistently to two species of *Amoeba* by Leidy (**11**) were mistaken by him for normal appendages and thus made the basis for a separate genus *Ouramoeba*, may belong here, the chains of fusoid segments shown in the figures of *O. botulicauda* Leidy (**11**, *pl. 9, figs. 13–17*) being especially suggestive of relationship. While the filamentous attachments (*Amoebophilus Korotneffi* Dang.) that led Korotneff (**10**) likewise to propose a new genus of *Amoebae, Longicauda*, show somewhat less resemblance to the known members of the group in question, the possibility of relationship is yet not to be excluded; and the same situation ob-

tains apparently in regard to the different outgrowths (*Amoebophilus caudatus* Dang.) which Penard (**14**) figured and discussed as being fungi attached to *Amoeba nobilis* Pen. and *Amoeba vespertilio* Pen. Dangeard's figures of the fungus outgrowths on his *Pelomyxa vorax* to which he (**5**) applied the binomial *Amoebophilus Penardi* reveal *Leptomitus*-like constrictions through which a condition not greatly unlike that evident in immature spore chains of *Cochlonema verrucosum* and *Bdellospora helicoides* is brought about.

In view of the extensive study devoted to *Amoebae* for many decades the paucity of literary references to fungi possibly assignable to the series under consideration might seem remarkable, especially as much more definite descriptions and records of parasites belonging to such relatively difficult chytridiaceous genera as *Sphaerita* and *Nucleophaga* are available in some number. The explanation for this paucity very probably lies in the fact that protozoölogists have very largely kept or cultivated in water the animals studied by them. All of the five newly described fungi as well as the various allied predacious forms are pronouncedly terrestrial in their asexual reproduction, if not also in their sexual reproduction and biological adaptations. It appears very doubtful whether the adhesion of conidium or filament in *Endocochlus asteroides, Bdellospora helicoides* and *Zoopage phanera* necessary for penetration can occur when the host animal is bathed in free liquid water. Even if vegetative development were provided for under aquatic conditions, the production of conidia, so important in the multiplication of these fungi, would generally be meager as evidently it can proceed only in the air. In cultures consisting of irrigated decaying plant materials, one of the predacious forms figured earlier (**9**, *fig. 2*) has often been seen to extend its mycelium, partly submerged, partly floating, some distance from the solid substratum, but its conidia even then were always formed in the air on floating filaments. Indeed, appearances suggest that in nature the special function of the curious empty appendages, whose presence on the conidia of this species as on those of several of its predacious allies constitutes a feature anomalous among the Phycomycetes, might be to give the buoyancy necessary to prevent

submergence in local accumulations of water following rains or heavy dews.

If aquatic conditions while facilitating observation are unfavorable for normal development of the fungi under discussion, terrestrial conditions attaching to natural substrata like moist soil, leaf mold, decaying plant remains and excrement of animals, favor abundant normal development but impose serious impediments to observation. The rather meager and scattered conidial apparatus is usually neither very conspicuous in the much more luxuriant growth of saprophytic forms often surrounding it, nor disposed in a manner to make easy the manipulations entailed in transfer to a microscopic preparation; and when successfully mounted shows little or nothing to distinguish it from that of commonplace Hyphomycetes. With sexual apparatus, vegetative thallus and all evidence of its curious parasitism concealed in an opaque substratum, *Endocochlus asteroides* might with good fortune be referred perhaps to the genus *Acladium*; while *Cochlonema verrucosum, C. dolichosporum, Bdellospora helicoides* and *Zoopage phanera* might find places among the unsifted species compiled in *Fusidium*. It is therefore in these Hyphomycetous genera and in *Oospora*, closely similar to *Fusidium*, that the fungi herein described and forms strictly congeneric with them most likely need to be looked for in the literature; though my own searches here have so far yielded no immediately useful information.

In this connection it may not be amiss to direct attention to the general similarity of conidial development in *Cochlonema, Bdellospora* and *Zoopage* to that described earlier (**6**) as occurring in *Actinomyces*, a genus formerly often included in *Oospora*. This similarity gains in suggestiveness from the circumstance that the vegetative mycelium characteristic of *Actinomyces* with its sparse septation largely if not exclusively consequent to protoplasmic degeneration in aging portions, or to withdrawal of protoplasm into younger ramifications, conforms rather well in fundamental design with that of the Phycomycetes. The extraordinary delicacy of the mycelium in *Actinomyces*, while very little reminiscent of the more familiar types of fungi among the Oömycetes and Zygomycetes, is, as has been mentioned, matched in the extremely slender hyphae of the minute *Amoeba*-capturing forms concerning

whose close relationship in the Phycomycetes to the three catenulate genera herein described, there can be no reasonable doubt. To be sure the conidia of none of the more minute *Amoeba*-capturing forms now known are either catenulate or of dimensions at all comparable in smallness to the filaments from which they are produced; and in the known members of the catenulate genera neither the filamentous parts nor the conidia can be regarded as especially small. Yet when, as in the present instance, an obviously natural group embodies extreme delicacy of mycelium in some members, and in others formation of catenulate conidia not greatly exceeding in diameter the hyphae on which they are borne, the possibility of its relationship to forms wherein both these features are combined deserves consideration. Until the affinities of *Actinomyces* are definitely revealed through the discovery of a convincing sexual stage—the "Vierhyphensporen" described by Lieske (**13**) are far from impressive when viewed in such character—the morphological resemblances just noted would seem to compare more than favorably with the similarities that have long been cited as justifying the relegation of the genus to the higher bacteria.

For the time being the disposition of the group embracing the four newly described genera and the forms obviously kindred to them presents a more immediate problem. The similarities and possible homologies suggested more particularly in a comparison between *Bdellospora helicoides* and *Syncephalis nodosa* would seem to betoken articulation with the Mucorales through the Piptocephalidaceae. On the other hand the sturdy nematode-capturing form (**8**, *fig. 8*), in the moderate and often even meager development of its vegetative or predacious mycelium, in the habitual migration of its protoplasm from old hyphae to newly proliferated branches, in the large dimensions of its conidia, and in the repetitional production of secondary conidia from primary ones, reveals features suggestive of some sort of interdigitation with the Entomophthorales. What would seem to make for a provoking analogy with the latter order is apparent also in the semi-predacious behavior, as it were, of many species of *Empusa* in fixing their enfeebled insect hosts to the substratum through the production of adhesive rhizoids. A taxonomic position somewhere between the

Mucorales and the Entomophthorales is thus indicated for the group, which it is believed deserves recognition as a separate family, to be designated with perhaps tolerable appropriateness as the Zoopagaceae. Indeed, there is some reason to believe that further study of fungi destructive to terricolous microscopic animal life under approximately natural conditions on suitable transparent solid subtrata, will, without impairing the distinctiveness of the group, bring to light a range in morphological diversity and a plenitude of species commensurate in the Phycomycetes with the taxonomic scope of a suborder or of an order rather than with that of a family.

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EXPLANATION OF PLATES

PLATE 1

A, A well developed and active specimen of *Amoeba terricola* I, showing: *a*, penetration by an infective germ tube from an adhering conidium of *Endocochlus asteroides*; *b*, development of a globular body at tip of infective germ tube; *c*, separation of the globular body or young vegetative thallus from the evacuated germ tube, the former remaining within the animal, the latter being expelled; *d–i*, young thalli within the animal in increasingly advanced stages of development; *n*, nucleus of host animal; *v*, contractile vacuole of host; × 1000. *B*, An animal with two vegetative thalli which have given rise to three pairs of zygophores and a conidiiferous hypha shown in three sections,—*a* and *b* representing corresponding points on these sections, and the dotted line indicating the point of emergence from the substratum; × 500. *C*, An animal with a single well developed thallus that has given rise to a branching conidiiferous hypha with conidia in various stages of development, the branches being shown in sections,—*a–g* indicating corresponding points on these sections, and the dotted line the point of emergence from the substratum; × 500.

PLATE 2

A, A specimen of *Amoeba terricola* I, shortly before succumbing to infection from two well developed thalli of *Endocochlus asteroides*. *B*, An animal soon after its death from the three or four thalli of various sizes massed together in its interior. *C*, Remains of an animal containing eight thalli of *E. asteroides* of small and moderate sizes which have become evacuated in giving rise to seven zygosporangia, *a–g*. *D*, Wrinkled pellicle of an animal enveloping and concealing the membranes of the thalli which have produced four zygospores, *a–d*, approaching maturity. *E*, Remains of an animal showing eight mature stellate zygospores, each loosely enveloped by the collapsing zygosporangial membrane, *a–g*. *F*, A pair of zygophoric hyphae with a nearly fully grown zygosporangium produced on a short prolongation from the junction. *G*, A portion of conidiiferous hypha showing *a*, an early stage, and *b*, a later stage in the lateral proliferation of a conidium. *H*, An intermediate stage in conidial development. *I*, An advanced stage in conidial development. *J*, Mature conidia, *a–f*, showing variation in shape and size. Magnification × 1000 throughout.

PLATE 3

A, Pellicle of an unidentified *Amoeba* containing an empty conidium of *Cochlonema dolichosporum* and attached to it, the thallus, also empty, produced from it. *B*, Conidia of *Cochlonema dolichosporum*, showing variation in size and shape, arrangement in chain, and appendaged condition at

late maturity. *C*, Pellicle of a specimen of *Amoeba sphaeronucleolus* enveloping a single thallus of *Cochlonema verrucosum* with the conidium from which it had origin still attached, and showing the basal portions of the conidiiferous hyphae to which it gave rise. *D*, *Amoeba sphaeronucleolus* about at point of death from the parasitism of three internal thalli of *Cochlonema verrucosum*, two of which have given rise to normal hyphae, while the third, through somewhat abnormal development, has developed externally a thallus-like swollen hypha. *E*, A dying specimen of *Amoeba sphaeronucleolus*, showing in addition to its two contractile vacuoles and its nucleus, three thalli of *Cochlonema verrucosum*, two of which have each given rise to an immature conidial chain shown in sections, whereof *a*, *b* and *c* represent corresponding points. *F*, Mature conidia of *Cochlonema verrucosum*. *G*, Remains of a specimen of *Amoeba sphaeronucleolus* with two zygosporangia of *Cochlonema verrucosum*, one still growing, the other containing a nearly mature zygospore. Magnification × 1000 throughout.

PLATE 4

A, Same as Plate 3, *C*, but showing the four conidial chains, *a–d*, in their entirety,—*b* and *c* being still in course of development, *a* and *d* being approximately mature; and the points of emergence of the conidiiferous hyphae from substratum being indicated by dotted lines; × 500. *B*, Same as Plate 3, *A*, but showing the asexual reproductive apparatus of *Cochlonema dolichosporum*, consisting of three conidial chains, *a–c*, in its entirety; points of emergence of conidiiferous hyphae into the air being indicated by dotted lines; the considerable lengths of the portions of conidiiferous filaments submerged in the substratum being due to the depth at which the host animal succumbed; × 500.

PLATE 5

A, Conidia of *Bdellospora helicoides*, showing variation in size and shape. *B*, A specimen of *Amoeba terricola* II with nine infections from separate conidia of *Bdellospora helicoides*, the separate conidia being designated alphabetically *a–i* approximately in the order of their respective stages of development into swollen vegetative bodies; *n*, nucleus of animal; *v*, contracting vacuole. *C*, Another specimen of *Amoeba terricola* II, still alive, on which have developed six swollen spore bodies, which have given rise to four zygosporangia, *a–d*; one pair of the bodies, through branching of their respective sexual hyphae having given rise to two pairs of zygophores. *D–H*, Approximately mature zygosporangia and zygospores of *Bdellospora helicoides*, together with portions of the zygophores. *I*, Remains of a specimen of *Amoeba terricola* II and of vegetative bodies of *Bdellospora helicoides* from which have been produced eleven zygosporangia of which eight, *a–h*, contain each a normal mature zygospore, while the remaining three, *i–k*, are empty as a result of internal degeneration. Magnification × 1000 throughout.

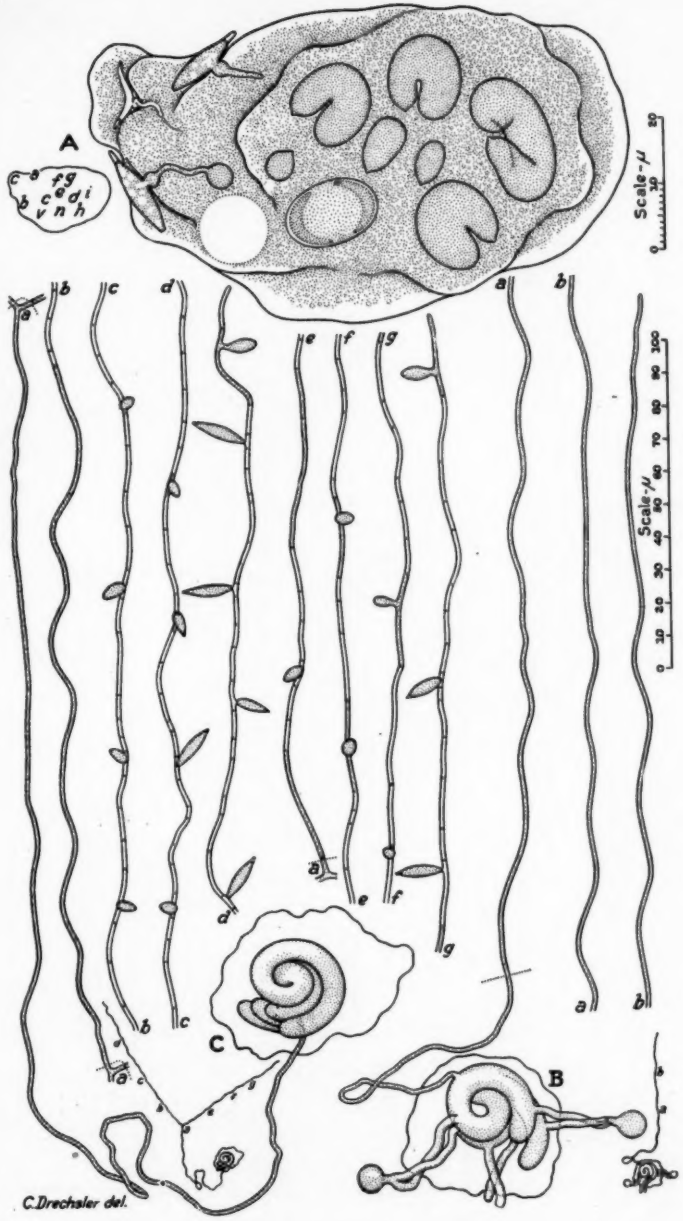
PLATE 6

A large specimen of *Amoeba terricola* II about to succumb to four infections from *Bdellospora helicoides*, the four swollen spore bodies having

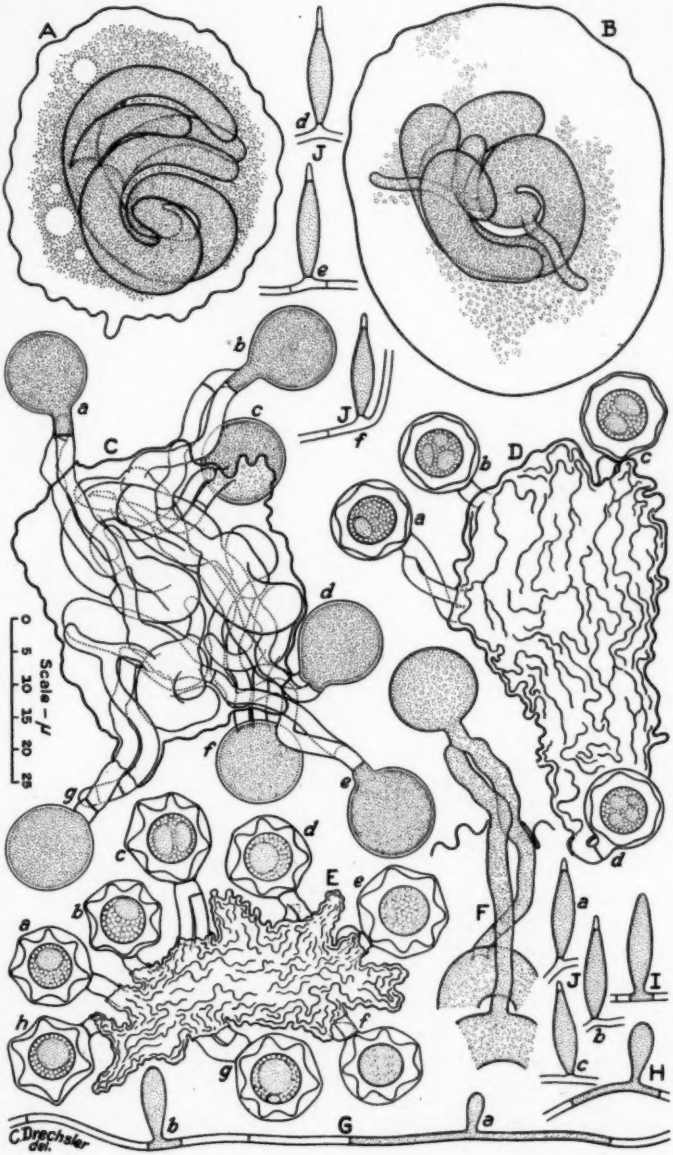
given rise to hyphae *A*, *B*, *C* and *D*, on which have been or are being produced chains of conidia *a–d*, *a–d*, *a–b* and *a* respectively; of these chains *A*, *b* and *D*, *a* are continuous and therefore immature. The dotted lines at *A* and *C* and the lower dotted lines at *B* and *D* indicate the points of emergence of the hyphae from the substratum, the upper dotted lines at *B* and *D* the divisions between prostrate and erect portions. × 500.

PLATE 7

A, Four specimens, *a*, *b*, *c* and *d*, of *Amoeba terricola* III captured by the branched hyphae, *e* and *h*, of *Zoopage phanera*, showing the stalked botryoid haustoria of the fungus; hyphae *f* and *g*, perhaps also adhering to animal but without having produced haustoria, bear respectively one and two erect conidiiferous branches with long chains of conidia of which from lack of space only the lowermost individuals are shown; in the animal *d* is shown its nucleus, and the same structure from a healthy specimen is shown in *n*; × 500. *B*, Two hyphae, bearing three conidial chains *a–c* (shown only in part from lack of space) and producing on diclinous sexual branches two zygosporangia, *d* and *e*, shown at early stages of development; × 500. *C–H*, Sexual apparatus of *Zoopage phanera* showing diclinous origin of zygophores, inconstancy of septation during earlier stages in development of fusion product, and the frequently contorted condition of one of the zygophores; × 1000. *I–M*, Approximately mature zygospores, each within its collapsing zygosporangial membrane; × 1000. *N*, *O*, Sexual apparatus, with a germ tube from a conidium functioning directly as a zygophore; × 1000.



ENDOCOCHLUS ASTEROIDES



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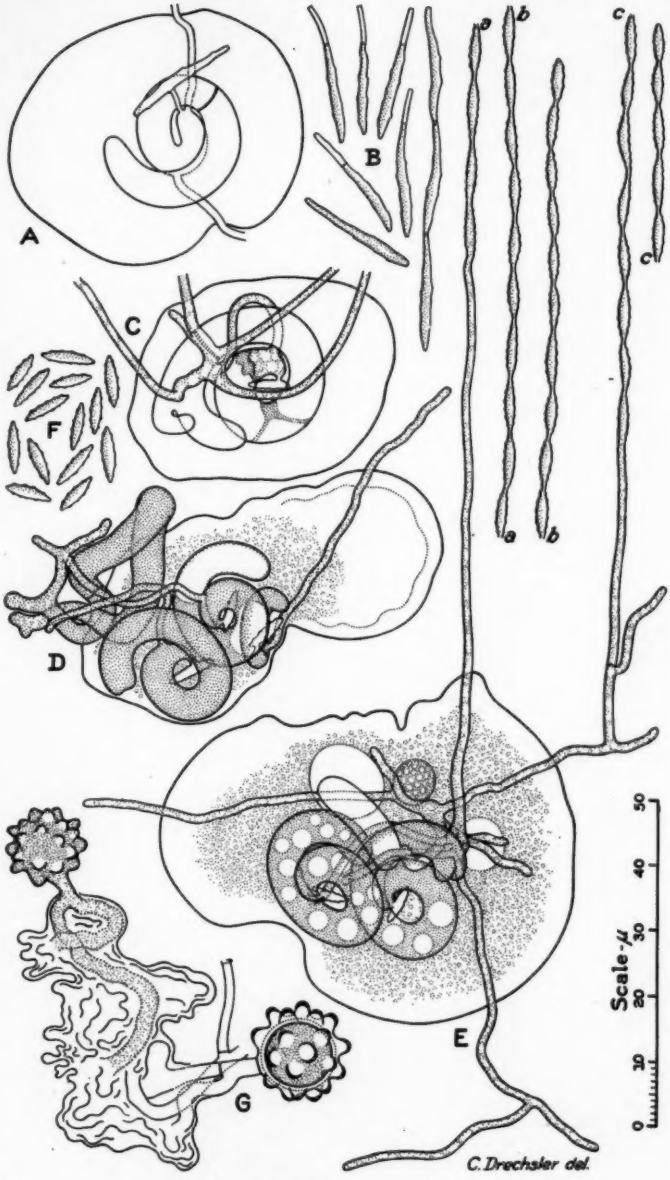
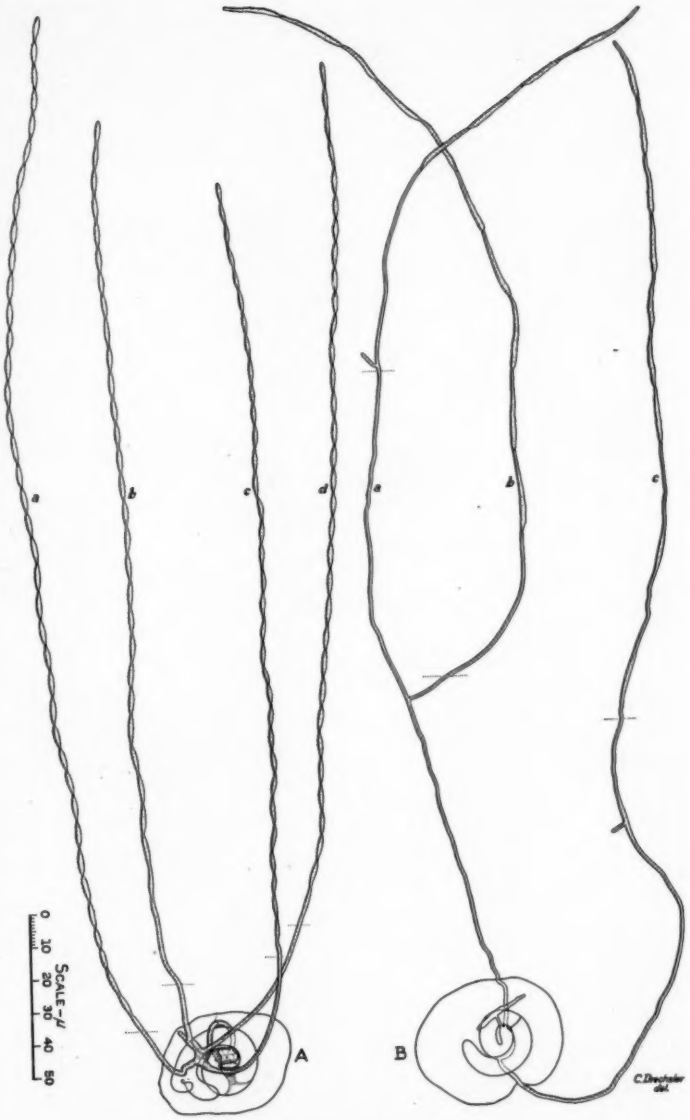
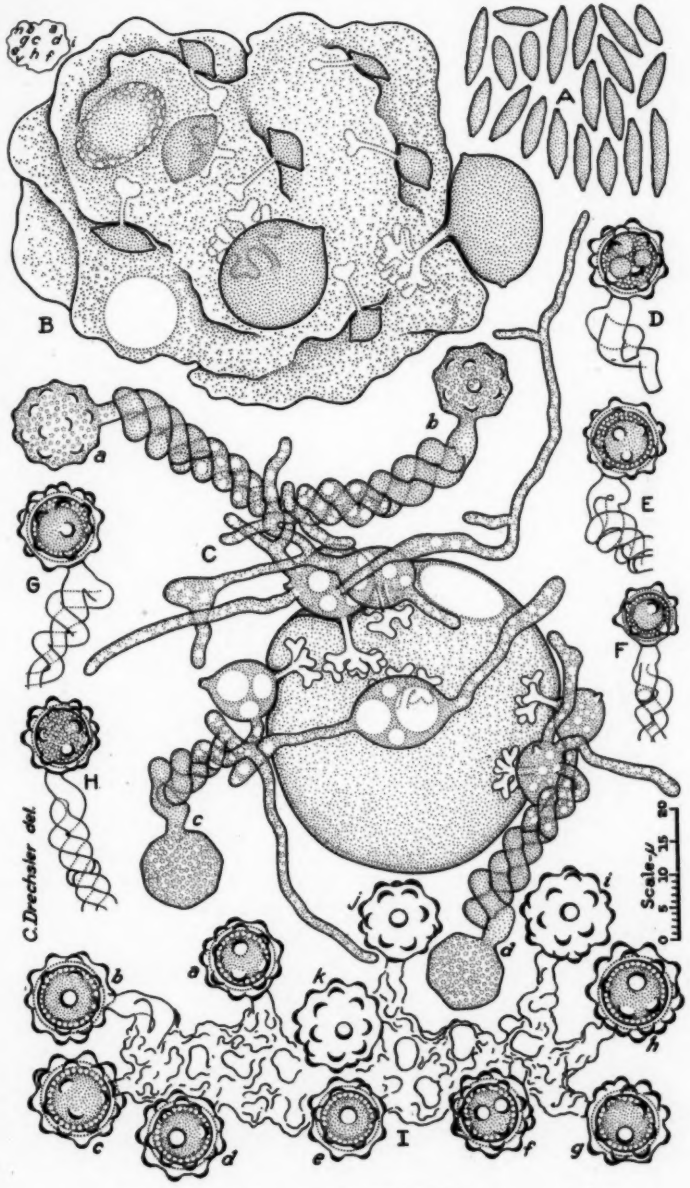


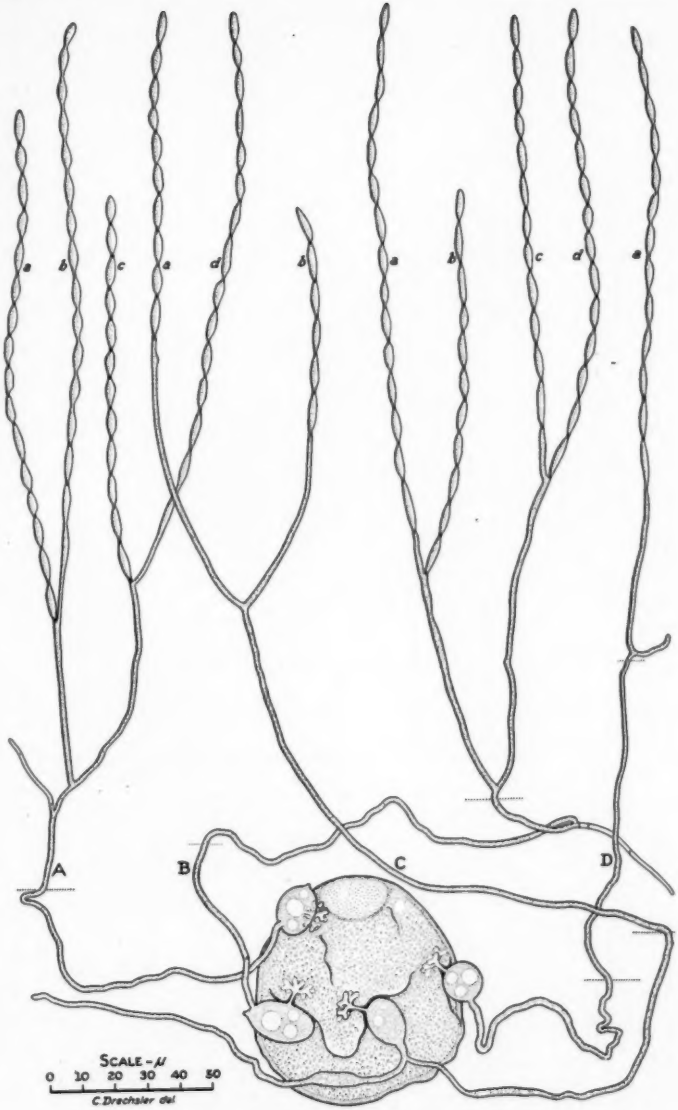
A–B. COCHLONEMA DOLICHOSPORUM
C–G. COCHLONEMA VERRUCOSUM



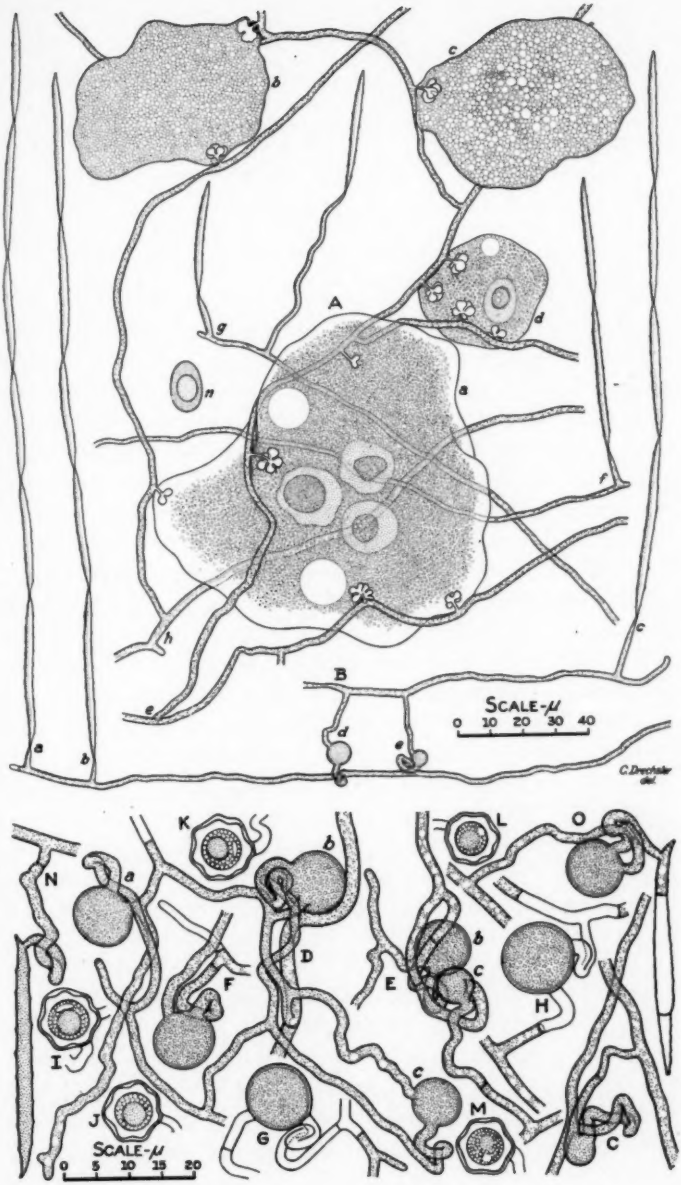
A. COCHLONEMA VERRUCOSUM
B. COCHLONEMA DOLICHOSPORUM



BDELLOSPORA HELICOIDES



BDELLOSPORA HELICOIDES



ZOOPAGE PHANERA

CYTOLOGICAL STUDIES IN THE TREMELLACEAE II. EXIDIA[1]

ROY M. WHELDEN

(WITH PLATES 8–11)

It is most surprising in view of the fact that *Exidia* is generally recognized as the most abundant genus of Tremellaceae, that there has been so little published on its cytology. The reason for this soon becomes apparent when one undertakes such a study, for, as Neuhoff has stated, (Transl.) "For cytological treatment with the fixing solutions and stains used the species of *Exidia* are only slightly suitable since for the most part they take up dyes very poorly, so that after I began my researches with members of this genus, I had nothing to record in the first months except failure." With this statement the writer does indeed agree, for it was only after months of trials resulting in almost complete failure, that the successful methods explained fully in a previous paper (**5**) were developed. Even then failure to obtain satisfactory sections was not infrequent. Eventually, however, the writer obtained some three hundred collections of fruit-bodies of the various species of *Exidia* occurring in New England, *i.e.*, *E. glandulosa* (Bull.) Fries, *E. recisa* Ditm., *E. nucleata* (Schw.) Burt, and *E. saccharina* Fries, from which, using material collected and fixed in the field, many hundreds of satisfactory sections were cut and studied. From this material, the cytological facts here presented have been drawn.

HISTORICAL BACKGROUND

In the earlier papers one finds occasionally information other than taxonomic; for example, Sautermeister (**4**), in 1876, noted that there occurred on the upper surface of *Exidia recisa* Fries tubercles of filaments and paraphyses, and also that in succession on the same stroma first mature acrogenous spores (conidia) and

[1] Contribution No. 133 from the Laboratories of Cryptogamic Botany of Harvard University.

later "Schlauchfrucht" developed. Several years later, in 1888, Brefeld (**1**) studied the members of the genus and on the basis of the straight cylindrical form of the secondary spores of *Exidia saccharina* separated this as *Ulocolla* from the other species, which have sickle-shaped secondary spores.

Ten years later Juel (**2**), in 1898, studied various fungi, among them *Exidia truncata* Fries. While he could not find the preliminary stages, he did note the presence in the basidium of a fusion nucleus which presently divided in two reduction divisions to form four small nuclei located near the center of the basidium.

Recently Neuhoff (**3**), in 1924, undertook to make a comprehensive study of the various Tremellaceae, among them *Exidia*. In no case did he obtain a complete connected story of any species: in *Exidia glandulosa* and also in *E. truncata*, he elaborated on Brefeld's results, noting the uninucleate condition of the small secondary spores and also the formation of the primary mycelium with its uninucleate segments; in *E. repanda* he found little worthy of note other than the sole case of fusion between hyphae which he saw in any species of *Exidia*; in *E. saccharina* Fries he obtained more complete results, noting the presence of a transversely oriented spindle in the hypobasidium, and observing that nuclear migration from the latter through the epibasidia occurred with "no change in form," and finally that binucleated spores occur (his *figure 31* of *pl. 4* shows these to be two-celled).

With his paper cytological study of the species of *Exidia* seems to have lapsed. There is an imperative need for a more intensive study of the species in order that as complete a picture of the development as possible may be obtained.

EXIDIA

Four species of *Exidia*, all occurring rather commonly in New England, have been studied intensively in the present work. Of these species, three, *E. glandulosa* (Bull.) Fries, *E. recisa* Ditm. and *E. nucleata* (Schw.) Burt, are found very frequently on almost all broad-leaved woody plant stems and branches, while the fourth, *E. saccharina* Fries, appears to grow exclusively on the branches of *Pinus Strobus* L.

Habit

In the youngest condition the fruit-bodies of all four species are so closely similar as to be indistinguishable, and are hemispherical, smooth-surfaced, transparent greyish bodies which in section are composed entirely of branching, frequently anastomosing hyphae, 1–1.5 μ in diameter, densely protoplasmic and with binucleate segments (PLATE 9, FIG. 1c; PLATE 10, FIG. 1, 2; PLATE 11, FIG. 6, 7). Developing fruit-bodies very quickly gain that color which is characteristic of the mature fructification, reddish-brown to black in *E. glandulosa,* clear red-brown in *E. recisa,* Auburn brown [2] in *E. saccharina,* and Cinnamon Buff in *E. nucleata.*

With increasing size of fruit-body come certain slight but definite changes in the hyphal structure therein. In *E. glandulosa,* hyphal branching is relatively infrequent; the hyphal segments become quite elongate and frequently multinucleate due to rapid repetition of nuclear division (PLATE 8, FIG. 1); anastomoses occur frequently, the hyphal ends enlarging slightly during the process (PLATE 8, FIG. 2). Division of the small apparently structureless nuclei shows four very evident small chromosomes which split longitudinally; while not invariably so, such nuclear divisions are frequently noted as occurring in connection with clamp formation. Such nuclear divisions almost invariably occur in the hypha at the point from which the clamp forms. As the clamp develops, one or rarely both nuclei resulting from division migrate into the base of the clamp; only one nucleus passes through the clamp, however, and enters the adjoining hyphal segment, now separated from the next by a septum (PLATE 8, FIG. 3). Much the same condition obtains in the hyphae of *E. recisa,* except that they exhibit as a rule a much more twisted condition (PLATE 9, FIG. 1, 2). *E. saccharina* shows much more frequent anastomoses (PLATE 10, FIG. 5), almost invariably associated with nuclear division (PLATE 10, FIG. 4) and with apparent migration of nuclei from one hypha to the other (PLATE 10, FIG. 6); and also shows a peculiar "prong," devoid of protoplasm, projecting stiffly out from a clamp connection (PLATE 10, FIG. 3). In *E. nucleata,* however, a quite different structure is found; for here, a lateral branch

[2] Capitalized color names are from Ridgway's Color Standards and Nomenclature.

develops, pushing the main filament to one side and becoming cut off, leaving the main filament quite definitely angled. A second branching may occur in similar fashion but the original segment, no matter how many times angled, remains binucleate, for a considerable length of time (PLATE 11, FIG. 7). Anastomoses between distinct branches are generally frequent (PLATE 11, FIG. 5), and are usually unique in that one of the two tips becomes much enlarged, appearing more or less to surround the other (PLATE 11, FIG. 8). Clamp connections (PLATE 11, FIG. 2) as well as the "prongs" previously mentioned also occur in this species (PLATE 11, FIG. 4), as does another structural feature, the white "kernels" of calcium oxalate, around which is found a fairly compact weft of densely protoplasmic infrequently branched hyphae.

Should the fruit-bodies of the various species of *Exidia* be subjected to periods of desiccation at this stage of development, very characteristic surface changes appear. In *Exidia glandulosa* and *E. saccharina* the fruit-bodies lose most of the 95 per cent or more of water they contain and shrink to a dry leathery patch tightly pressed to the surface of the substratum. Sections show that the hyphal tips at the surface of the fruit-body have become coarse, empty, thick-walled objects which branch frequently and are interlaced to form a tight network of hyphae spreading laterally over the entire surface (PLATE 8, FIG. 36, 37; PLATE 9, FIG. 34, 35; PLATE 10, FIG. 39). Fruit-bodies of *E. recisa* differ in remaining rigidly projecting from the substratum in much the same shape as when wet; internal structure accompanying this drying out shows the ends becoming thick-walled and devoid of protoplasm, but remaining separate and somewhat perpendicular to the surface rather than forming a thick interlacing layer (PLATE 9, FIG. 35). In *E. nucleata,* finally, relatively slight modification occurs on drying, the surface hyphae branching more frequently, becoming full of drops of "oil" and of small diameter, but not developing thick walls (PLATE 11, FIG. 34).

Basidia

The hymenium covers the exposed surface of the fruit-body in all species excepting *E. recisa* in which it is more or less confined

to the upper surface of the unilobed fruit-body. The basidia form either at the surface or, in those fruit-bodies which have developed thick-walled hyphae, beneath these. The hyphal tips which are to develop into basidia first become distinguishable through their densely protoplasmic content and somewhat swollen appearance (PLATE 8, FIG. 4, 5; PLATE 9, FIG. 3–6; PLATE 10, FIG. 7–11; PLATE 11, FIG. 9, 10). Into these slightly swollen tips move the two small nuclei, which may occupy almost every conceivable relative position in the basidium, and which have a diameter of 0.7–0.8 μ in all species except *E. nucleata,* in which it is 0.3 μ. Here they approach one the other, frequently losing their spherical shape as they do and becoming elliptical or even fusiform. These variations in shape seem not to be due to any mutual attraction. As the two primary basidial nuclei come together, rapid enlargement of the hypobasidium initial begins; this enlargement shows certain rather definite specific characters. In *E. glandulosa,* the hypobasidium becomes blunt, somewhat coarse and with uniformly dense protoplasm, which abruptly thins at the point where the basal septum will presently separate the basidium from the hypha (PLATE 8, FIG. 5). In *E. recisa,* the hypobasidium, almost from the first, becomes a fusiform body, broadest in the middle where the two nuclei are located (PLATE 9, FIG. 5). *E. saccharina* shows a hypobasidium with sides for a considerable time almost parallel, and with the apex containing a dense cap of protoplasm (PLATE 10, FIG. 8). In contrast to the other three species, the hypobasidium of *E. nucleata,* which is at the tip of a long, almost straight, slender stalk, enlarges to a subspherical body considerably smaller than that in the other three species (PLATE 11, FIG. 10).

As this enlargement begins, the two nuclei fuse to form the fusion nucleus which differs strikingly from the previously mentioned small nuclei of the mycelium. Almost from its inception, this fusion nucleus shows a very definite organization quite in contrast to the mycelial nuclei in which only the nucleolus can be distinguished clearly. In the fusion nucleus, not only is the nucleolus, comprising the two fused nucleoli, distinct, but also the chromatin material is definitely arranged into distinct linear patches (PLATE 8, FIG. 6, 8–10; PLATE 9, FIG. 7, 9; PLATE 10, FIG. 12–16; PLATE 11, FIG. 11, 13–15). This chromatin is seen more and

more clearly to be located just inside the membrane of the now rapidly enlarging nucleus; the nucleolus is also located on the periphery (PLATE 8, FIG. 8). In *E. glandulosa,* the nucleolus may very rarely become extruded to a distance from two to four microns from the nuclear surface.

As the fusion nucleus reaches its maximum size, the linear patches of chromatin, now definitely distinguishable as eight in number and not arranged in a continuous spireme, gradually contract longitudinally, while at the same time, the disintegration of the nucleolus takes place. Generally the nucleolus has entirely disappeared by the time contraction is half completed (PLATE 8, FIG. 10, 15, 16). Contraction ends with the formation of eight nearly spherical or short cylindrical chromosomes about 0.5 μ in diameter (PLATE 8, FIG. 12; PLATE 9, FIG. 8, 10; PLATE 10, FIG. 17; PLATE 11, FIG. 12, 16). Throughout this development the nucleus remains near the middle of the hypobasidium. At the time when chromosome formation is completed, the hypobasidium has reached its maximum size, but so great is the range of dimensions of this body that little importance can be attached to a mere mention of dimensions, which in *E. glandulosa* range from 15×7, 11×5.5, 20×4, 13×7, 9.5×7 μ, etc., with an average from a hundred random measurements, of 18×6 μ (in *E. recisa* similar measurements give an average of 12.1×6.7 μ; in *E. saccharina,* 10.3×6.1 μ, while in *E. nucleata,* where much greater uniformity exists, the dimensions are 7×5 μ).

With chromosome formation the nuclear membrane disappears, leaving the mass of chromosomes usually compactly clumped near the center of the hypobasidium, while at the same time the transversely oriented and somewhat vague spindle appears (PLATE 8, FIG. 11; PLATE 9, FIG. 11; PLATE 10, FIG. 18; PLATE 11, FIG. 19). Definitely recognizable centrosomes were never seen during the present studies. To each of the two poles of the indefinite spindle four of the eight chromosomes migrate, thus definitely indicating as the reduction division the first to occur in the hypobasidium. Only in very rare instances is the first division equational, and in the few cases of this nature seen, all in *E. glandulosa,* there resulted a longitudinally directed spindle (PLATE 8, FIG. 13, 14).

The two daughter nuclei, fairly uniform in size for each species,

and from 1–1.5 μ in diameter, occupy positions more or less midway between the base and tip of the hypobasidium (PLATE 8, FIG. 19; PLATE 9, FIG. 14; PLATE 11, FIG. 17). Normally not until they are completely formed after division does the basal septum cut off the basidium from the hypha of which it was the tip. Greater irregularity attaches to the time of formation of the first longitudinal septum, which may develop, usually basipetally, before the basal septum is formed, especially in *E. glandulosa* and *E. saccharina,* or after, as in *E. recisa,* or with the greatest irregularity, as in *E. nucleata.* The formation of the second longitudinal septum follows directly after the second nuclear division in the hypobasidium.

This second nuclear division, almost always mitotic, except in those cases mentioned above, with four chromosomes apparent, normally occurs during or immediately after the formation of the first longitudinal septum (PLATE 8, FIG. 17, 18; PLATE 10, FIG. 19; PLATE 11, FIG. 18, 23). *E. recisa* is an exception in that both nuclear divisions frequently occur before septum formation begins (PLATE 9, FIG. 12, 13). Not infrequently the second division fails to occur, only two nuclei being formed and ultimately only two epibasidia (PLATE 8, FIG. 20; PLATE 11, FIG. 26, 27). The second division, like the first, is transverse to the long axis of the hypobasidium, with the result that the four final nuclei are all at about the same level near the middle of the hypobasidium (PLATE 10, FIG. 21, 22; PLATE 11, FIG. 25). The two divisions usually follow one another in rapid succession. In all species herein studied, the second nuclear division not infrequently occurs independently in the two nuclei, one nucleus often completing its division before that of the other begins (PLATE 9, FIG. 16, 17); indeed not infrequently only one nucleus divides, the result being a three nucleate hypobasidium, from which only three epibasidia will develop (PLATE 8, FIG. 34; PLATE 9, FIG. 18).

Epibasidia

The formation of the epibasidia seems to be more or less independent of the events occurring in the hypobasidium. While the mature structures show certain fairly constant specific differences, the method of formation is uniform in all species studied. The

first indication of epibasidial development is the simultaneous appearance of two, three, or usually four small hemispherical bulges of the outer end of the hypobasidium (PLATE 8, FIG. 17, 19; PLATE 10, FIG. 21; PLATE 11, FIG. 21, 22). The rate of development of the epibasidia arising from a single basidium is practically equal and so continues until they have extended to the surface of the "jelly" or beyond. Certain differences observable in the mature epibasidia are as follows: In *E. glandulosa,* they are slender, slightly twisting objects 16 to 30 μ long, whose diameter increases somewhat from the basal 0.8–1.6 μ to a maximum of 2.5 μ when they project rigidly out above the "jelly" surface (PLATE 8, FIG. 32, 34); in *E. recisa,* the epibasidia, often arising close together at the tip of the hypobasidium, are slender, nearly straight objects which extend rigidly 10–20 μ or more above the "jelly" surface, at the same time abruptly expanding from the basal diameter of about 1 μ to approximately 2 μ (PLATE 9, FIG. 19–26); in *E. saccharina,* they are coarse, slightly tortuous objects whose length is determined solely by the amount of "jelly" present in the fruitbody, and whose diameter varies from 1.5–2 μ until they emerge from the "jelly," when it may increase to somewhat more than 3 μ (PLATE 10, FIG. 23–26; 36); in *E. nucleata,* the nearly straight, rather short, chunky epibasidia maintain a nearly constant diameter from 1–1.5 μ even when they extend slightly above the "jelly" surface (PLATE 11, FIG. 24; 26–28).

When the epibasidia have reached the surface of the "jelly," the nuclei begin migrating individually or simultaneously from their approximately central position in the hypobasidium, becoming at the same time more and more elongated, until, in *E. glandulosa,* they are slightly more than three times as long as their diameter, now about 0.5 μ (PLATE 8, FIG. 32) (*E. nucleata* is an exception in having nuclear migration start soon after the inception of the epibasidia). Without further change the nuclei migrate into the epibasidia, and rapidly progress to its outer end, where a pronounced alteration in shape occurs, namely, a decided extension toward the sterigma, at the tip of which extension the nucleolus may frequently but not invariably be observed (PLATE 8, FIG. 21, 34; PLATE 9, FIG. 22–25; PLATE 10, FIG. 29). In rare cases, the

nucleus may round up in the epibasidium, seeming to remain in this condition for some time (PLATE 10, FIG. 28).

During the migration of the nucleus into the epibasidium, there has developed from the tip of the latter the sterigma, a slender tapering object, which in *E. recisa* may reach a length of 5 μ, but is generally much less (PLATE 8, FIG. 32; PLATE 10, FIG. 27). At the tip of the sterigma the spore initial first appears as a small spherical object which rapidly enlarges until it reaches the diameter of the mature spore, when elongation occurs (PLATE 8, FIG. 21–23; PLATE 9, FIG. 22–25; PLATE 10, FIG. 29, 30). As this elongation takes place the nucleus, now extremely attenuated, passes through the sterigma and into the spore (PLATE 8, FIG. 22–23; PLATE 9, FIG. 26–28; PLATE 10, FIG. 30–31; PLATE 11, FIG. 33). A trace of this nuclear elongation frequently remains even when the nucleus has practically reached its final location in the distal half of the spore (PLATE 8, FIG. 24).

As the nucleus enters the epibasidium, the protoplasmic content of the hypobasidium becomes very vacuolate; the protoplasm in the epibasidium becomes equally vacuolate after the passage of the nucleus, and when the latter enters the spore, practically all traces of protoplasm have disappeared in the basidium (PLATE 8, FIG. 34; PLATE 9, FIG. 30; PLATE 11, FIG. 33). As the spore elongates to its mature dimensions it too becomes more and more vacuolate (PLATE 8, FIG. 25, 26; PLATE 9, FIG. 29–31; PLATE 10, FIG. 32, 35, 37, 38; PLATE 11, FIG. 29, 30, 31).

Mature Spores and Germination

The spores are capable of remaining dormant but viable for long periods of time. Germination of the spores readily occurs at any time, either immediately after maturity or after a considerable time, if there be sufficient water present. (In one instance, spores of *E. glandulosa* were successfully germinated, nine months after they were collected, on a glass slide placed under a fruit-body.)

On germination of the spore, the nucleus, with four chromosomes present, divides at once, while simultaneously with the early stages of nuclear division there is formed either laterally or terminally one or two germ-tubes, into each of which there migrates

one of the very small daughter nuclei, the average diameter of which is 0.3–0.5 μ (PLATE 8, FIG. 27; PLATE 11, FIG. 31, 32). In the spore itself, there may be several successive nuclear divisions, after each of which septa will form to divide the spore into from two to four uninucleate segments (PLATE 8, FIG. 28–30). Each nucleus may migrate into a germ-tube, leaving an enucleated segment in the spore, or it may divide and only one of the resulting nuclei enter the tube; or several nuclei in succession may enter a single germ-tube. In any event, cross-walls will eventually form so that the densely protoplasmic germ-tube is divided into one to several usually sickle-shaped (in *E. saccharina* straight cylindrical) secondary spores, 1–2 μ in greatest diameter and 3–3.5 μ in length, into which the very small nucleus migrates (PLATE 8, FIG. 30, 31, 33). Rarely this nucleus divides there, and a cross-wall is formed to produce a two-celled secondary spore (PLATE 8, FIG. 33). All attempts to germinate these secondary spores failed.

At times certain exceptional developments are noted. Not infrequently and apparently caused by sudden wetting, following partial drying, a basidial initial starts growing into a single tube, into which each of the primary nuclei migrate without fusing. At other times coarse hyphal tips arising from the subhymenial hyphae project rigidly up through the hymenium; at times the whole exposed surface of the fruit-body is covered with these densely protoplasmic hyphal tips, which have been particularly noticed in *E. recisa* and *E. saccharina* (PLATE 9, FIG. 32, 33; PLATE 10, FIG. 34). Subsequent behavior indicates that these may be active in the formation of the "jelly" of the enlarging fruit-body.

DISCUSSION

The results brought out in this paper lead to the conclusion that the conflicting statements made by the few previous workers result from insufficient material. Certainly Neuhoff's emphasis on the scarcity of interhyphal fusions is not borne out by the present work; for fusions are of common occurrence in all species studied, particularly *E. glandulosa* and *E. saccharina*. In young fruit-bodies, and also in old ones in which disintegration of hyphae may be taking place, fusions are admittedly infrequent, but in actively growing fruit-bodies practically every section will show them.

As one follows the development of a young fruit-body, he sees the hyphal tips characteristic of rapidly enlarging bodies (PLATE 10, FIG. 33; PLATE 11, FIG. 1) giving place to the young basidia whose protoplasmic content is very dense. These seem never to have been described in this genus; perhaps because such basidial initials differ but little in various tremellaceous fungi. Nevertheless, except in the very earliest stage, certain quite noticeable differences are really apparent. The most conspicuous is that of shape, which ranges from bluntly club-shaped in *E. glandulosa* and *E. saccharina,* through fusiform in *E. recisa,* to long slender tips in *E. nucleata.* In addition to this, the conspicuous apical "cap" of protoplasm in *E. saccharina* is distinctive, as is also the frequent branching of the basidial hypha (PLATE 10, FIG. 15), a phenomenon rarely to be observed in the other three species studied.

These differences in shape are noticeable while the hypobasidium is still binucleate. As the two minute nuclei move together and fuse in the enlarging hypobasidium, these differences gradually diminish, and differences in size become apparent. While the relatively smaller hypobasidia of *E. nucleata* set it off sharply, the separation of the other species is only apparent when measurements of large numbers of basidia are tabulated.

As in the previously mentioned nuclear fusions, so in the present development of hypobasidium and included fusion nucleus, the few recorded facts are in the main correct. So far as the writer knows there is no detailed statement of any part of this development. As in the case of *Tremella,* here also a definite organization obtains in the fusion nucleus from the first. The chromatin material becomes organized in eight definite patches located more or less just beneath the nuclear membrane. In this region also is the rather large nucleolus which results from the fusion of the two minute nucleoli from the primary basidial nuclei. As the fusion nucleus approaches its maximum size, the nucleolus begins to disappear, and the prochromosomes to contract into the eight minute approximately spherical chromosomes. Coincident with this process, the nuclear membrane is lost, and there appears the transversely directed spindle, which as far as the present studies reveal, never stands out distinctly at any time.

The nuclear divisions of the mature fusion nucleus occur in

rapid succession, the daughter nuclei becoming definitely reorganized between them. The first of these divisions is almost invariably the reduction division. In the one observed case in which the reduction seems to come in the second division, the spindle of the first division was longitudinally directed in the hypobasidium. In general the four daughter nuclei are formed at approximately the same level, midway in the now mature hypobasidium: numerous exceptions to this may be observed, it is true, but their number is small when compared to that of normal arrangement.

In the formation of the septa which divide the hypobasidium, little occurs worthy of note. Relatively great variation in direction of septation is the only peculiarity. Equally lacking in noticeable variation is the earlier part of the formation of the epibasidia.

As these approach mature size, they are, in *E. glandulosa,* objects which definitely enlarge in diameter, and reach a length of 16–30 μ, which is much greater than that in the other three species; in these three, the epibasidia of *E. saccharina* with a diameter of 2–3 μ are relatively coarser than those of *E. nucleata,* with a diameter of 1.5 μ, and of *E. recisa,* which are generally the most slender of the three. The absolute dimensions of these epibasidia, and to a less degree, those of the hypobasidia, offer little of value in specific determination: for the length of the epibasidium is determined by the amount of "jelly" making up the bulk of the fruit-body; if the weather is wet, the "jelly" is most abundant and the epibasidia relatively longer than usual. No connection seems to exist between the divisions of the basidial nuclei and the time of formation of the epibasidia, although usually the second division takes place at about the beginning of the growth of the epibasidia.

That there is no change in shape of the nucleus as it migrates in the basidium has been stated by Neuhoff. While it is true that there is no conspicuous change, yet the writer has always noted a definite elongation apparent in the migrating nucleus; in a body so minute this may easily escape notice. However, as the nucleus approaches the tip of the epibasidium, and before it enters the sterigma, this elongation becomes very pronounced. While the minute size of the nucleus renders very difficult accurate observa-

tion of this point, the position of the nucleolus seems in no way to be determined by nuclear migration.

When the nucleus reaches the tip of the epibasidium, the spore borne thereon is usually well developed. The process of development leads first to the formation of a somewhat spherical object whose diameter is that of the mature spore; subsequently this spherical body elongates to form the typical bent cylindrical spore characteristic of all species of *Exidia*. The size of the spores offers very evident specific ranges more definitely than do any structures previously mentioned. Only infrequently do spores depart conspicuously in size from the average; *i.e.*, very small spores, obviously abnormal (PLATE 8, FIG. 35), may be formed during the later stages of desiccation.

The behavior of the mature spore on germination is quite unlike that noted in *Tremella*. In the species of *Exidia* studied, nuclear division always accompanies germination; following which, the spore becomes divided by the number of septations. It is interesting to note that these nuclear divisions frequently occur in the germ-tubes which develop singly from each of the segments of the spore. The minute size of the nuclei has made it impossible to work out the details of the process of division here. Secondary spores, sickle-shaped save in *E. saccharina*, where they are straight cylindrical, are now developed from the germ-tube. Into each of these a nucleus migrates to become the nucleus of the spore; not infrequently this nucleus, in those cases where the secondary spore becomes two-celled, then divides again. This is wholly in agreement with the facts recorded by Neuhoff as occurring in *E. glandulosa*. Further the germination of the secondary spores was not followed, since they failed to germinate under any conditions.

In addition to the differences in spore shape which separate species of *Exidia* from *Tremella*, and in the method of germination just noted, there is another very noticeable difference which appears when the fruit-body begins to dry. This is the formation in species of *Exidia* of the layer of thick-walled interlacing hyphae which form over the fruit-body. Lacking these to a considerable degree, *E. nucleata* is less sharply set off from *Tremella* than are the other species here studied.

SUMMARY

The cytology of the four species here studied, *Exidia glandulosa* (Bull.) Fries, *E. recisa* Ditm., *E. saccharina* Fries, and *E. nucleata* (Schw.) Burt, is remarkably uniform in all particulars.

Between the usually binucleate segments of the hyphae fusions frequently occur. Clamp connections are abundant, also.

Over the upper surface of the fruit-body the young hypobasidia, each binucleate, develop. In these, nuclei unite to form the fusion nucleus. As this enlarges, very definite organization is visible, the chromatin being clearly aggregated in eight linear patches, the prochromosomes, which contract to form the eight small chromosomes.

The first nuclear division in the basidium is the reduction division, which is followed by a second homotypic division. Subsequent to this division, the minute nuclei migrate through the epibasidia into the spore. Contrary to Neuhoff's report, the nucleus becomes definitely elongated during migration.

Nuclear division accompanies spore germination in *Exidia*. This is in contrast to the situation in *Tremella*, where no nuclear division occurs in the usual method of spore germination.

The formation of a layer of thick-walled interlacing hyphae over the surface of the drying fruit-body is characteristic of species of *Exidia*. This feature does not occur in species of *Tremella*.

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DESCRIPTION OF PLATES

PLATE 8, EXIDIA GLANDULOSA (BULL.) FRIES

In this and all other plates, all figures have been drawn with the aid of a camera lucida, at a magnification of 3800 ×, and subsequently reduced to about 0.3, *i.e.*, to a magnification of about 1150 ×. In addition, an absolute scale of dimension is included in all plates.

Fig. 1, hyphal segments in which the nuclei are dividing conjugately, each nucleus showing 4 chromosomes; 2, anastomosis of hyphal tips (*a*) appressed, slightly swollen tips; (*b*) clamp-like development of one tip around end of other; 3, clamp formation showing (*a*) nucleus dividing before entering the clamp, (*b*) uncommon condition in which one of the daughter nuclei migrated through the clamp before a cross-wall has formed, (*c*) daughter nucleus entering the clamp which passes around cross-wall of hypha, and (*d*) final condition; 4, formation of basidia showing various positions of the two primary basidial nuclei which in *b* and *c* are of the frequent fusiform shape; 5, 7, hypobasidium showing formation of a lateral branch; 6, 8, 9, the enlargement of the fusion nucleus with increasingly distinct prochromosomes; 10, 16, fusion nuclei from which the nucleolus has disappeared and with prochromosomes definitely formed; 11, the nucleus shows eight small chromosomes; 12, the eight prochromosomes have contracted until the chromosomes are distinct; 13, first division with its axis parallel to the longitudinal axis; 14, the second division following fig. 13; 15, prochromosomes contracting and nucleolus disintegrating; 17, the nuclei in the second division, two epibasidia forming apically, and the earliest indication of longitudinal septum; 18, a slightly later stage of the division of the nuclei and the formation of the septum; 19, the two epibasidial initials appearing; 20, an early stage in epibasidial formation; 21, an epibasidium in which the elongated nucleus is approaching the slender sterigma at the tip of which the spore is forming; 22, tip showing the entrance of the nucleus into the spore; 23, tip bearing nearly mature spore; nucleus still connected with the sterigma by a slender strand; 24, the same, with the nucleus still showing a slender strand extending towards the sterigma; 25, nearly mature spore at tip of slender sterigma; 26, mature spore showing the nucleus in early prophase; 27, mature spore with lateral germ-tube and nucleus in metaphase; 28, 29, 30, 33, spores showing septate condition subsequent to nuclear division and stages in the development of the small falcate conidia; 31, falcate conidium showing the single minute nucleus; 32, vacuolate hypobasidium showing the nuclei passing from the mature epibasidia into the maturing spores; 35, abnormally small spore formed on a drying fruit-body; 36, apical portion of irregularly branched thick-walled hypha forming over surface of drying fruit-body; 37, stages in the development of these thick-walled hyphae showing nuclear disintegration and increasing vacuolation of protoplasm.

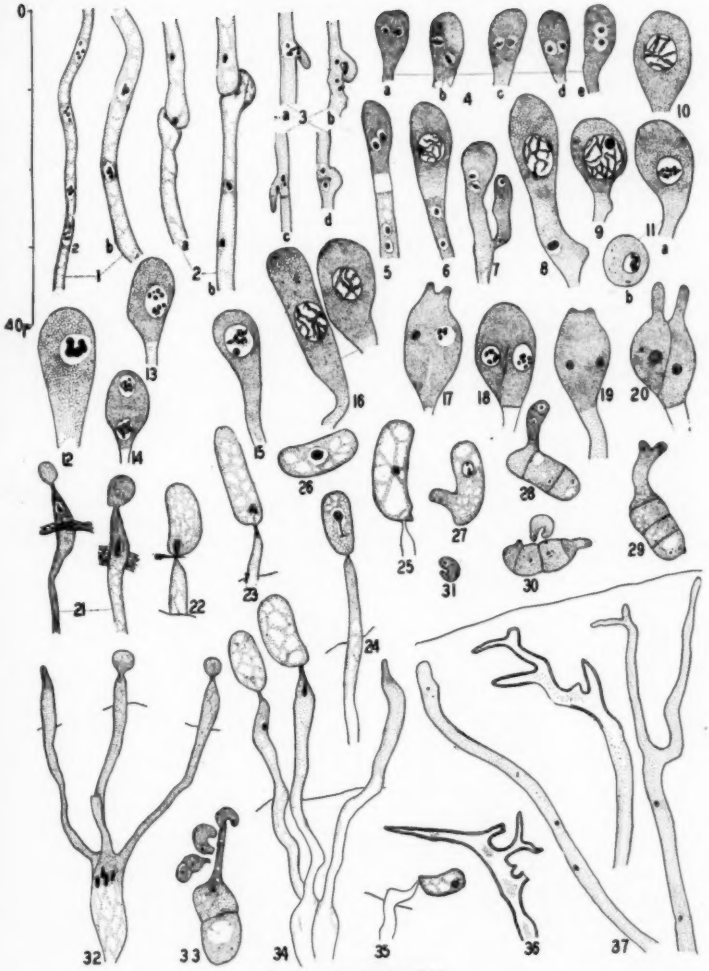
PLATE 9, EXIDIA RECISA DITM.

Fig. 1, *a*, somewhat enlarged fusing apices of hyphae; *b*, nucleus dividing in clamp; *c*, "loop" clamp at end of binucleate segment of hypha; 2, clamp

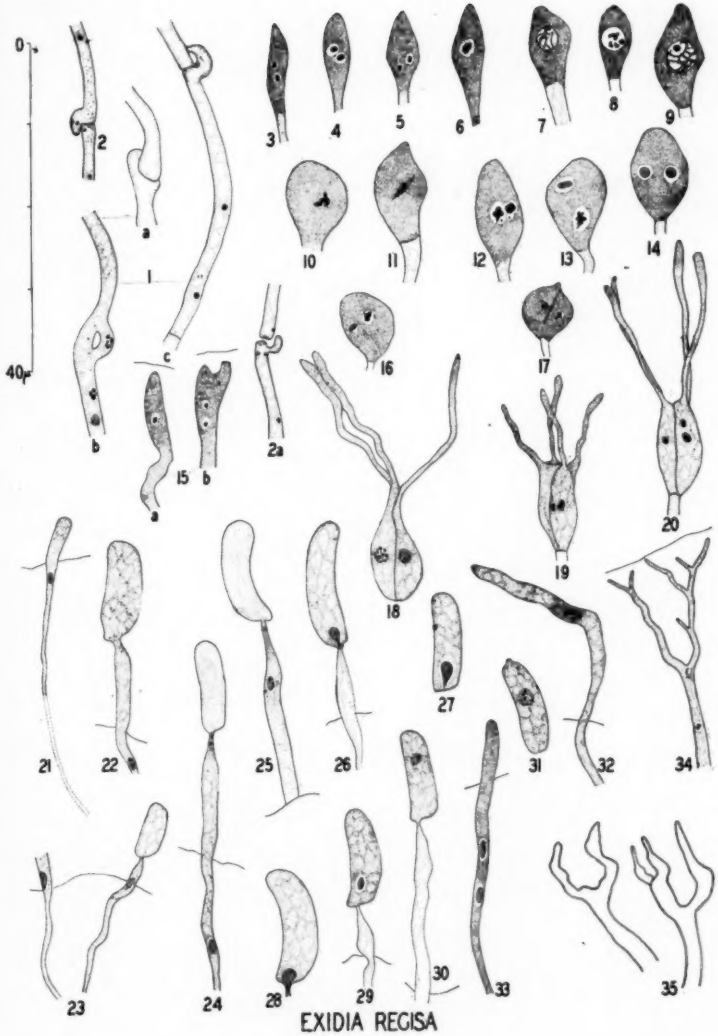
forming and receiving a migrating nucleus; 3, 4, 5, fusiform hypobasidia showing two nuclei in each; 6, hypobasidium showing the nuclei just fusing; 7, an enlarging fusion nucleus and the basal septum forming; 8, eight chromosomes in the fusion nucleus, in which the prominent nucleolus is still evident; 9, the mature fusion nucleus with evident nucleolus and eight elongate prochromosomes; 10, eight small chromosomes clumped together; 11, a diagonally oriented spindle; 12, two daughter nuclei dividing simultaneously; 13, two daughter nuclei dividing consecutively; 14, two daughter nuclei; 15, abnormal hyphal tips occurring among the basidia; 16, 17, small hypobasidia showing the formation of the first longitudinal septum; 18, hypobasidium showing three epibasidia originating close together; 19, four epibasidia arising apically; 20, the beginning of nuclear migration toward the epibasidia; 21, mature epibasidium showing the elongate small nucleus migrating the apex; 22, epibasidium showing the elongate nucleus approaching the tip on which a nearly mature spore has formed; 23, 24, 25, epibasidia showing successive stages in the development of the spore; 26, epibasidium tip bearing the nearly mature spore into which the nucleus is just migrating; 27, mature spore containing the pear-shaped nucleus migrating toward the center; 28, mature spore into which the nucleus is just passing from the sterigma; 29, 30, mature spores showing collapsed epibasidia on which they have formed; 31, mature spore showing the nucleus in early prophase; 32, 33, sterile hyphae which project rigidly above the surface of the fruit-body; 34, branching hypha which will become thick-walled when the fruit-body dries; 35, thick-walled hyphae which form over the surface of the drying fruit-body.

PLATE 10, EXIDIA SACCHARINA FRIES

Fig. 1, binucleate hyphal segment; 2, hyphal segment showing the nuclei in early prophase; 3, clamp showing "prong" projecting outward; 4, hyphal branch showing the dividing nuclei beneath; 5, anastomosing hyphal ends; 6, hyphae joined by a connecting strand at each end of which a nucleus is dividing; 7, young hypobasidium showing the two nuclei and protoplasmic "cap"; 8, a lateral branch developing from the stalk; 9, basal septum forming; 10, two nuclei just fusing; 11, forming laterally on a hypha; 12, nearly mature, showing the large fusion nucleus; 13, fusion nucleus with definite prochromosomes; 14, stage in the development of a lateral hypobasidium, later than fig. 8; 15, a complete hyphal tip showing laterally developed hypobasidia; 16, mature fusion nucleus; 17, eight chromosomes densely massed; 18, transverse spindle; 19, simultaneously dividing daughter nuclei separated by the first longitudinal septum; 20, abnormal hypobasidium showing the four daughter nuclei at about the same level and two epibasidia developing laterally; 21, normal hypobasidium showing four daughter nuclei at about the same level and the four apically forming epibasidia; 22, transverse sections of hypobasidium showing four daughter nuclei and the first longitudinal septum; 23, four epibasidia well formed prior to the second nuclear division; 24, one nucleus in division and two of the four well-developed epibasidia (the other two cut off in sectioning); 25, nuclei just entering the bases of the blunt epibasidia; 26, nuclei starting to migrate; 27, epibasidium tip tapering gradually into the sterigma; 28, nucleus below the sterigma;



EXIDIA GLANDULOSA



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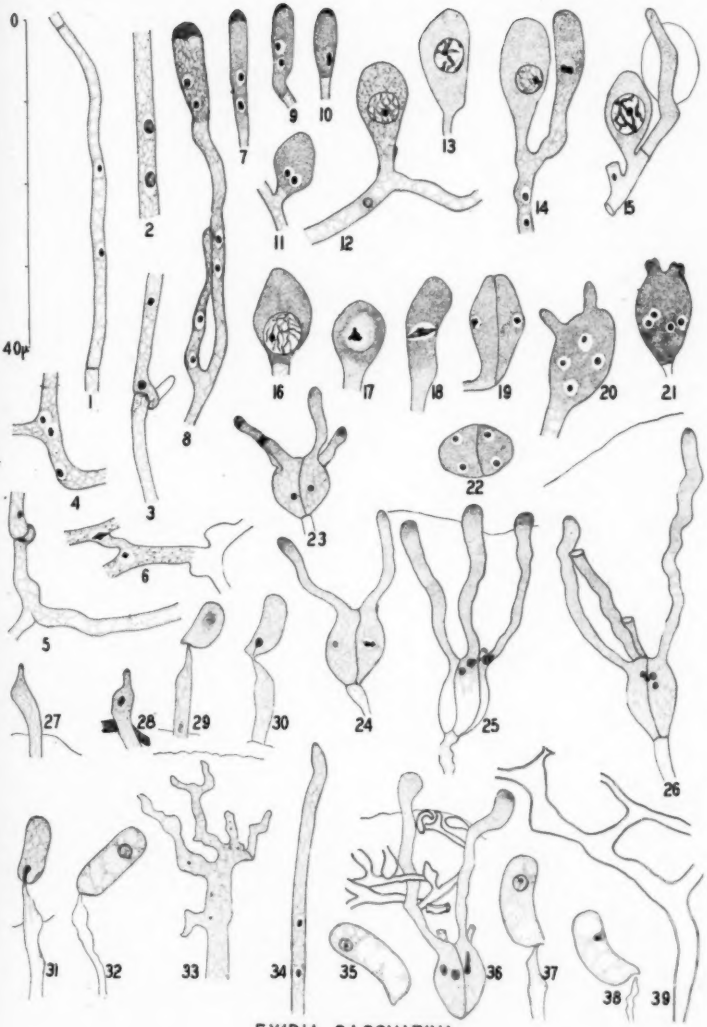

EXIDIA SAGGHARINA

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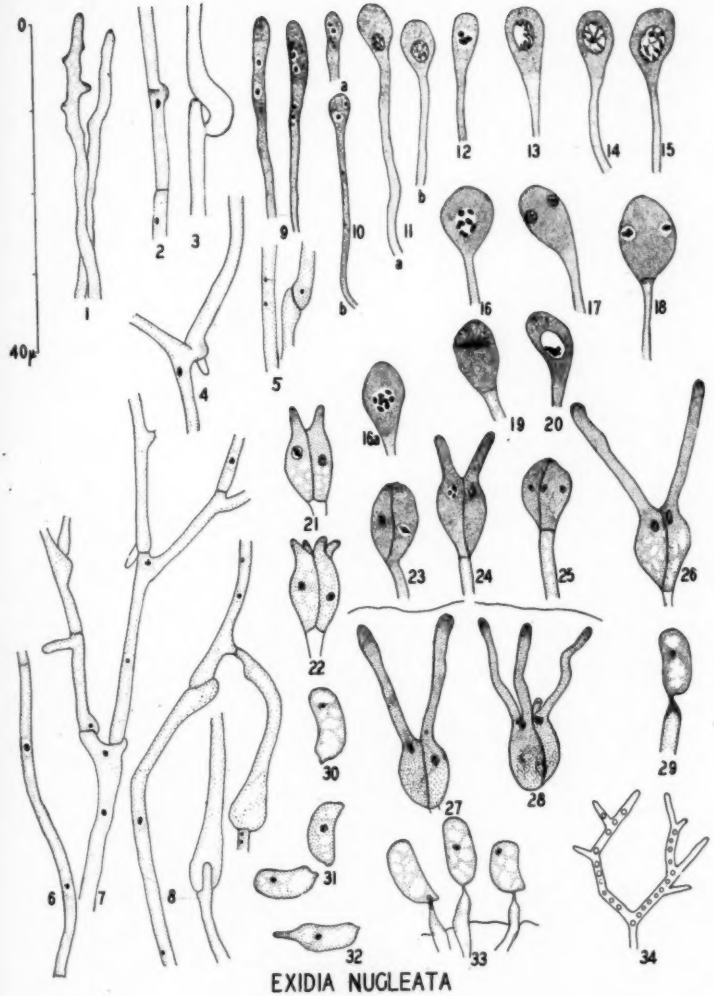

EXIDIA NUCLEATA

29, epibasidial tip on which the partly mature spore has formed; 30, nucleus from which a slender strand extends back to the sterigma; 31, nucleus just entering the spore; 32, mature spore on the collapsed epibasidium; 33, branched sterile hypha showing numerous disintegrating nuclei; 34, unbranched sterile hypha containing two nuclei; 35, mature spore showing vacuolate protoplasm and nucleus near distal ends; 36, epibasidia pushing up through the interlacing thick-walled hyphae; 37, mature spore on collapsing epibasidial tip; 38, mature spore showing vacuolate content and dividing nucleus; 39, portion of the thick-walled hyphae which form over the surface of the drying fruit-body.

PLATE 11, EXIDIA NUCLEATA (SCHW.) BURT.

Fig. 1, hyphal tips in young fruit-body; 2, segments of mycelium showing a single nucleus in each; 3, apical anastomosis showing loop-like formation; 4, hyphal branch showing "prong"; 5, terminal anastomosis of hyphae; 6, normal binucleate segment of mycelium; 7, branching hypha in fruit-body showing all the nuclei dividing; 8, anastomosis showing one hyphal tip much swollen; 9, 10, young hypobasidia showing two nuclei and long slender stalk; 11, hypobasidium showing the enlarging fusion nucleus; 12, nucleus in metaphase; 13, massing of nuclear content at one side; 14, 15, mature fusion nucleus with eight prochromosomes; 16, eight distinct chromosomes; 17, two daughter nuclei near the surface; 18, daughter nuclei dividing simultaneously; 19, transverse spindle of the meiotic division; 20, chromosomes all massed at one side; 21, 23, only one daughter nuclei dividing; 22, four wide-spreading epibasidia forming apically; 24, one daughter nucleus dividing while the other is migrating toward the base of an epibasidium; 25, four small nuclei; 26, 27, two nuclei migrating to the bases of the two epibasidia; 28, four epibasidia each receiving a migrating nucleus; 29, nearly mature spore at tip of the epibasidium; 30, extremely vacuolate content; 31, nucleus dividing; 32, apical germ-tube forming; 33, three epibasidia bearing nearly mature spores, one of which is just receiving the nucleus through the sterigma; 34, branched hyphal tip full of drops of oil-like substance.

MORPHOLOGY OF POLYTHRINCIUM, CAUSING SOOTY BLOTCH OF CLOVER

FREDERICK A. WOLF [1]

(WITH 5 TEXT FIGURES)

In 1910, the writer first attempted to isolate the fungus that causes the sooty blotch disease of clover, but was unsuccessful. Repeated attempts have been made, without success, in subsequent years. Apparently similar failures have rewarded the efforts of all other investigators, among whom are Killian (**5**), Bayliss-Elliott and Stanfield (**1**), and Horsfall (**3**), who have studied this fungus. It has been necessary, because this organism is so refractory in this regard, to confine the study of its morphology and development to such phases as could be ascertained from observations made in the field and from microscopic examinations of material collected at intervals throughout the year. The findings that have resulted are not in accord, in certain features, with those of others, and are herein recorded as a contribution to a better understanding of the structure of this unusual fungus.

SUSCEPTS

The sooty blotch fungus is widely prevalent throughout North America and Europe, and apparently is capable of attacking any of the species of *Trifolium*. No one has attempted studies to determine the suscept range, but apparently one and the same species of fungus is responsible for the disease on all species of clover. The writer has found no morphological differences in specimens of the conidial stage collected on red clover, white clover, alsike clover, and crimson clover, growing close together. Spegazzini (Mus. Nac. Buenos Aires Anal. **3**: 437, 1911) ascribed

[1] Contribution from the Cryptogamic Laboratories of Harvard University, 119. The writer is grateful to W. H. Weston, Jr., and D. H. Linder for help in interpreting the microscopic preparations and for their suggestions and criticisms.

the name *Polythrincium Trifolii* var. *platensis* to an organism on *Trifolium platense* in Argentina. The differences that he noted do not appear, however, to warrant the retention of this varietal designation. The following enumeration of suscept species is drawn from the records of the Plant Disease Survey, which show that the fungus has been widely collected within the United States, and from the compilations of Oudemans (Enumeratio Syst. Fung. **1–5** vol. 1919–1924), which show that it is represented in the exsiccati of such European mycologists as Cooke, Desmazieres, Fuckel, Rabenhorst, Roumeguère, Plowright, Saccardo, and de Thümen. It has been reported to occur on the following species: *Trifolium agrarium, T. alpestre, T. arvense, T. elegans, T. filiforme, T. fragiferum, T. incarnatum, T. lappaceum, T. medium, T. minus, T. Molineri, T. montanum, T. platense, T. pratense, T. procumbens, T. pseudobadum, T. reflexum, T. rubens, T. repens, T. scabrum, T. spadiceum, T. stellarum, T. striatum, T. tomentosum,* and *T. Wormskioldii.*

HISTORY OF THE FUNGUS

The sooty blotch fungus of clover is polymolphic, possessing a conidial stage, a spermogonial stage, and a perithecial stage, and for this reason, in part, it has been variously named. In 1801, it was first described in its spermogonial stage, by Persoon (Syn. Fung. p. 30, 1801), as "*Sphaeria Trifolii*: atra parua, magnitudine varia, caespitulo inaequali rugoso interne subpulverulento. Habitat autumno in foliis adhuc viridibus *Trifolii repentis.* ½–1 lin. lata. Sphaerulae farctae, intus albicantes." Neither Persoon nor the other mycologists of that period regarded spermogonia as male structures. In 1816, Schmidt and Kunze (Deutschl. Schwämme, no. *121*) first described the conidial stage as *Polythrincium Trifolii,* as follows: "Thallus e floccis caespitosis, erectis simplicibus multiseptatis. Sporidia didyma inspersa. Hypogenum in *Trifolio pratensi* aliisque." In the following year they (Kunze and Schmidt, Myc. Hefte **1**: 13–15. 1917) republished this description and included certain additional observations, notably that the fungus not only occurs on red clover, as shown in their specimen no. *121,* but also on *Trifolium alpestre* and *T. fragiferum,* that they had often noted it in the environs of Leipzig, and that botanists had sent them specimens as *Sphaeria Trifolii* of Persoon.

Fries (Syst. Myc. **2**: 435. 1923) suggested that the ascigerous stage should be a species of *Dothidea* but did not designate the clover organism as *Dothidea Trifolii* until 1849 (Summa Veg. Scand. p. 387. 1849). Even at that time he was unable to describe the ascigerous stage because, as he states, "asci hactenus frustra quaesiti" (the asci have thus far been sought in vain).

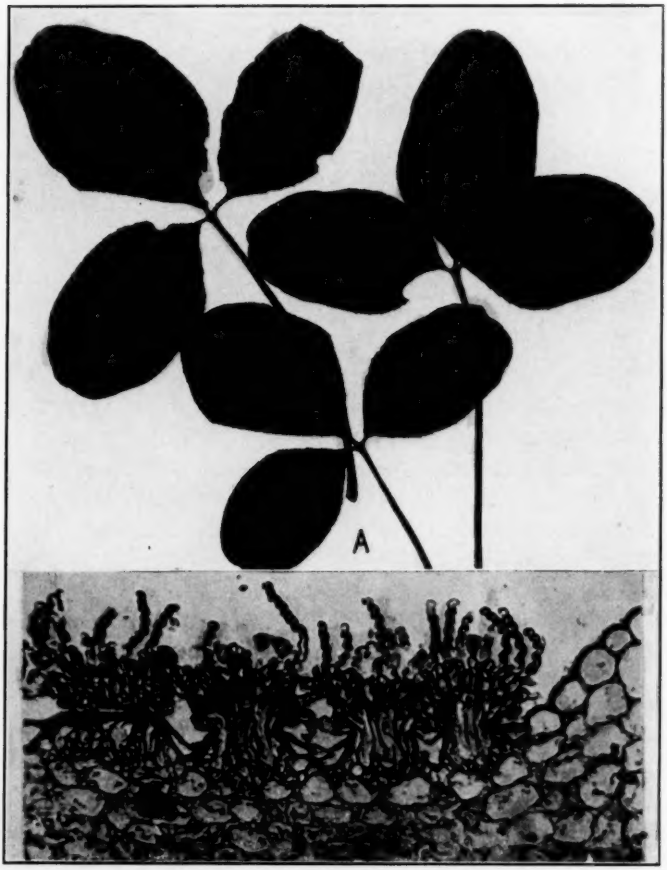

FIG. 1. A, The *Polythrincium* or conidial stage of the sooty blotch fungus of clover; B, Microphotograph of pustules of *Polythrincium* in sections cut perpendicular to the surface of the leaf.

Meanwhile Greville (Scot. Crypt. Botany **4**: 216, *4 figs.* 1826) employed Schmidt and Kunze's name, *Polythrincium Trifolii,* for the fungus that he noted to be of common occurrence in Scotland. The illustrations that accompany his account misinterpret its microscopic features and show that the conidiophores are articulately moniliform. They are also misinterpreted, as will be shown later in this report, by Corda (Ic. Fung. **3**: 10, *pl. 2, fig. 25*), whose illustrations have been copied into many taxonomic works and textbooks.

In 1869, Fuckel (Symb. Myc. p. 218, 1869) transferred *Dothidea Trifolii* (Pers.) Fries to *Phyllachora Trifolii* (Pers.), with *Polythrincium Trifolii* Schm. & Kunze and *Sphaeria Trifolii*-Pers. as synonyms, yet he pointed out that he had not seen the perithecial stage ("Fungus ascophorum nondum vidi"). This transfer met the approval of Saccardo (Syll. Fung. **2**: 613. 1883) for the reason, as he states, that the fungus has the general habit of *Phyllachora.*

Cooke (Grevillea **13**: 63. 1884–1885), in 1884, reported the presence of a specimen in the Berkeley Herbarium that possessed clavate asci bearing elliptical, continuous, hyaline ascospores, 10–12 × 5 μ. These specimens were reexamined by Bayliss-Elliott and Stanfield (**1**) but they were unable to verify Cooke's observation. In 1905, Clevenger (**2**) recorded ascospores of similar appearance, although slightly smaller, in specimens on *Trifolium Wormskioldii,* and identified the fungus as *Phyllachora Trifolii* (Pers.) Fuckel. These specimens are the basis of Theissen and Sydow's new species, *Phyllachora umbilicata* (**7**, p. 510), but they stated that none of the material that they examined possessed mature ascospores.

Traverso (**8**), in 1903, described the spermogonial stage as a pycnidial fungus, *Placosphaeria Trifolii* (Pers.), associated with *Polythrincium Trifolii* ("socio plerumque *Polythrincio Trifolii*").

Killian (**5**), in France, was the first investigator to find the perithecial stage of the clover pathogen in mature condition. He found that the ascospores are bicellular, and referred the fungus to *Plowrightia Trifolii* (Pers.) because *Phyllachora* has unicellular ascospores. From investigations conducted independently and at the same time in England, Bayliss-Elliott and Stanfield (**1**)

found that the ascospores are 2-celled, and assigned the organism to *Dothidella Trifolii* (Pers.) since the genus *Dothidella* has priority over *Plowrightia*.

DEVELOPMENT OF THE PATHOGEN

In the usual course of events in the developmental cycle of the sooty blotch fungus the ascospores constitute the inoculum for the primary infections in spring. This has been shown to be the case in France from the observations of Killian (**5**), in England, from the observations of Bayliss-Elliott and Stanfield (**1**), and appears, from the writer's observations, to be the case in the vicinity of Cambridge, Mass. A collection of mature perithecia was made on the campus of Mount Holyoke College, South Hadley, Mass., on May 12, 1934, and at this time there was no evidence there of the conidial stage. In North Carolina, however, the conidial stage has been found to be produced at any time throughout the entire year, even during the winter months, but it is most abundant in early spring and late autumn. Nevertheless mature perithecia may be found in North Carolina in late April and early May on leaves that had succumbed during the previous fall.

Conidial stage. The conidial stage appears as punctiform, olive-brown pustules or stromata that protrude prominently from the lower surface of the leaves. At first there may be no evidence of infection on the upper surface but gradually the areas above the pustules become pale green. The pustules may remain discrete and scattered or may become closely crowded and eventually so numerous as to cover over most of the leaf surface (FIG. 1, A). Each stroma arises subepidermally as a cushion of brown pseudoparenchymatous cells. As this stromatic tissue increases certain cells at its surface elongate perpendicular to the leaf surface and form a compact fascicle of a few to approximately 30 foot-like cells upon which the conidiophores are to be borne (FIG. 1, B and FIG. 4, 1). These foot-like cells extend to the surface by rupturing the epidermis, and each bears one or more conidiophores (FIG. 4, 1 and 4, 3). The conidiophores diverge distally in a broom-like fashion (FIG. 1, B). They are non-septate and are wavy in outline.

The conidial stage derives its name from the peculiar form of

the conidiophores. This form is the result of an unusual type of sympodial branching arising from the fact that each conidiophore eventually bears and sheds one at a time a series of conidia.

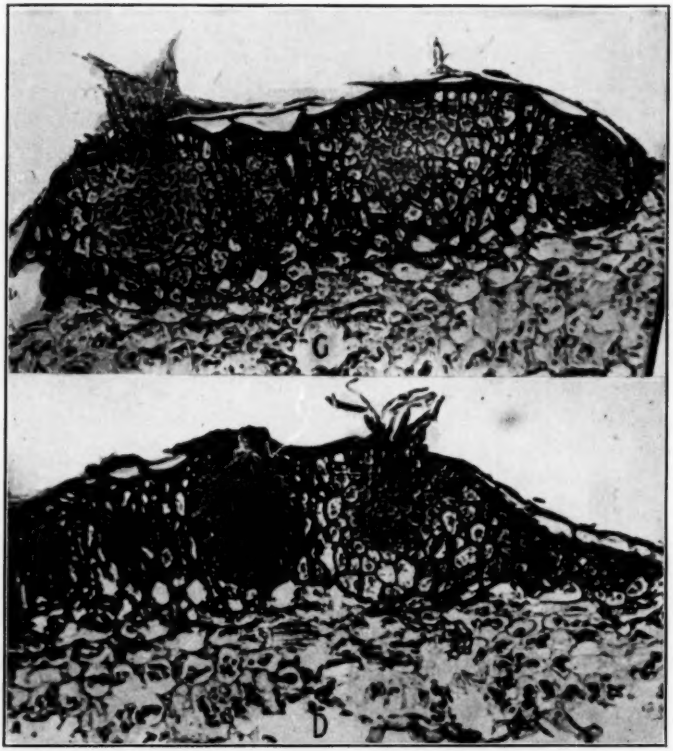

FIG. 2. C, Stroma of *Cymadothea Trifolii* in vertical section showing, at the right, a spermogonium; at the left, a perithecial fundament. Numerous spermatia occur in the mucilaginous matrix that invests the trichogynes; D, Vertical section of a stroma. A group of trichogynes project from the top of the perithecial fundament, at the right; the spermogonium is shown in median section at the left.

Young conidiophores are at first straight. Conidia are borne apically, and after each one is delimited the conidiophore continues to elongate. A point lateral to the point of attachment of the conidium marks the place at which growth of the conidiophore is

to be renewed. In elongating the tip of the conidiophore is deflected because of the firmness with which the conidium is attached at its rather broad base. The last-formed conidium is therefore dislodged as the result of the lateral pressure exerted upon it by the elongating conidiophore. Circular scars on the conidiophores mark the points from which the conidia were dislodged. These scars are normally unilateral and not spirally arranged (FIG. 3 and 4, 3). They appear as a series of thickened, brown pads when viewed in profile and as rings when viewed from in front. Apparently abstriction of the conidia proceeds slowly and in a manner represented by the closing of an iris diaphragm, as indicated by the small, central ring that marks the less thickened wall near the center of the scar. The conidiophores are thus definitely geniculate and not articulately moniliform, as shown by Greville (l. c.), nodose, as shown by Corda (l. c.), nor helical, as described by Killian (**5**).

The conidia (FIG. 4, 2) are not seen in position usually in paraffine sections since the conidiophores are directed downward and the conidia are normally shed as they mature. They may be found occasionally, however, attached either to the tip of the conidiophore or to its lateral wall (FIG. 4, 3). The cell walls of the conidial stromata and of the basal cells of the conidiophores are brown and thick, and their content is little stained by Haidenhain's iron alum hematoxylin. The walls of the older parts of the conidiophores are brown and firm, that of the elongating tips, thin and pale yellowish brown, but they contain an abundance of stainable material.

It has been impossible at any time to secure germination of more than a small proportion of the conidia in water, and growth of the germ-tube soon ceases (FIG. 4, 4), which observation accords with that of all other investigators.

Spermogonial stage. During autumn, the stromatic cushions that bore the conidiophores or others that arise from the intercellular mycelium (FIG. 5, 7) become the stromata that produce the spermogonia. They thus arise as a lateral expansion of the conidial stromata, as shown in FIG. 2, C, D, and 5, 5, or else as separate entities. These stromata extend to the exterior from the lower leaf surface. They vary in size up to a millimeter in

diameter and consist of large, compact, thick-walled, brown cells. They are plane above with a papillate surface, each papilla marking the orifice of a spermogonium. The spermogonia are spheri-

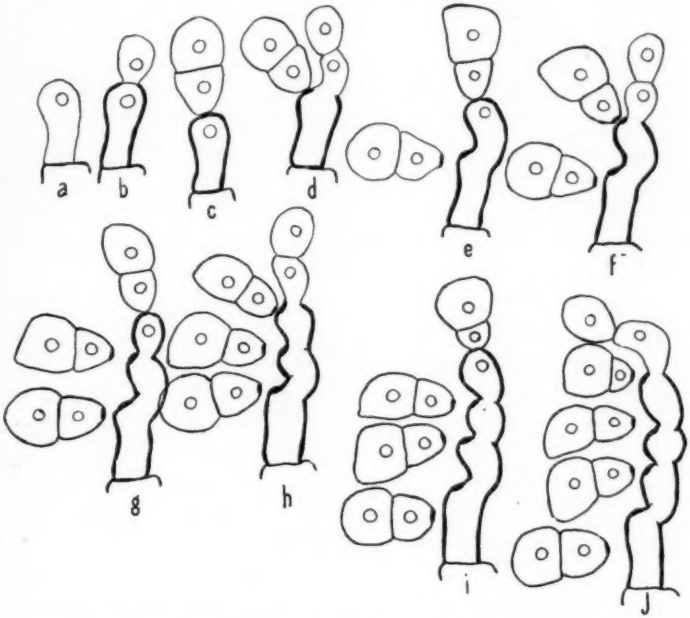

FIG. 3. Diagrams showing stages in the production of a series of conidia on a single conidiophore. a, Young, erect conidiophore; b, Young conidiophore from which a conidium is being abstricted; c, Mature conidium formed terminally; d, Elongation of the conidiophore, formation of a new conidium, and dislodging of the first-formed conidium; e, Two fully formed conidia and a lateral scar that marks the point at which the lower conidium was attached; f, g, h, i, j, Successive stages that show the repeated elongation of the conidiophore, dislodgment of the conidia, and the formation of new conidia apically.

cal to flask-shaped bodies in whose interior spermatia, 3–5 × 1.5–2.0 μ, are formed. The spermatia arise from parietal, spermatiferous cells that are ampulliform (FIG. 5, 8). They are formed in basipetal succession in such profusion that the entire spermogonium becomes filled, and they are forced out of the orifice in pale, mucilaginous droplets. This exudate may spread over the

surface of the stroma and on drying appear as a thin crust (FIG. 2, C and 5, 5). It is this stage of the fungus, as has previously been stated, that was first described by Persoon as *Sphaeria Trifolii*. Killian (**5**) regards it as the pycnidial stage that is responsible for the dissemination of the fungus during fall and early winter. Bayliss-Elliott and Stanfield (**1**) also regard it as a pycnidial stage, and it is described by Traverso (**8**) as the pycnidial fungus *Placosphaeria Trifolii*. Bayliss-Elliott and Stanfield (**1**) state that the pycnospores germinate by budding or by the formation of germ tubes, and present evidence that pycnospores produce infection. The writer has not been able to secure their germination, and feels that there is abundant evidence that they function as spermatia.

Ascigerous stage. The initials of the perithecia arise within the same stromata that bear the spermogonia and their initiation is coincident with that of the spermogonia. They appear at this time as locular, spherical masses of thin-walled, deeply-staining, uninucleate cells, embedded within the stromata. Some of the cells are somewhat larger than the others and become more deeply stained with Haidenhain's iron alum hematoxylin. Such cells are interpreted to be portions of the numerous, septate archicarps. In serial sections they can be traced to trichogynes, ranging in number from a few to 30 or 40, that extend from the surface of each perithecial initial (FIG. 2, C, D, and 5, 5). The numerous trichogynes and ascogones in each perithecial fundament are similar to the condition noted in *Polystigma rubrum* by Trifonova (**9**). The spermatial ooze adheres in quantity to these trichogynes (FIG. 2, C). Water appears to be essential for the transfer of spermatia from the orifice of the spermogonium to the trichogynes. Unless they are brought into contact with the trichogynes further development of the perithecia is apparently impossible. This is indicated by the fact that few or no mature perithecia are developed on leaves collected when the perithecia are just beginning to form, if such leaves are kept in the laboratory and are thus removed from the possibility of becoming wetted by dews and rains. The writer's failure to recognize the necessity of water for spermatization undoubtedly accounts for the lack of perithecia in material collected, in several seasons, at times when the conidia were

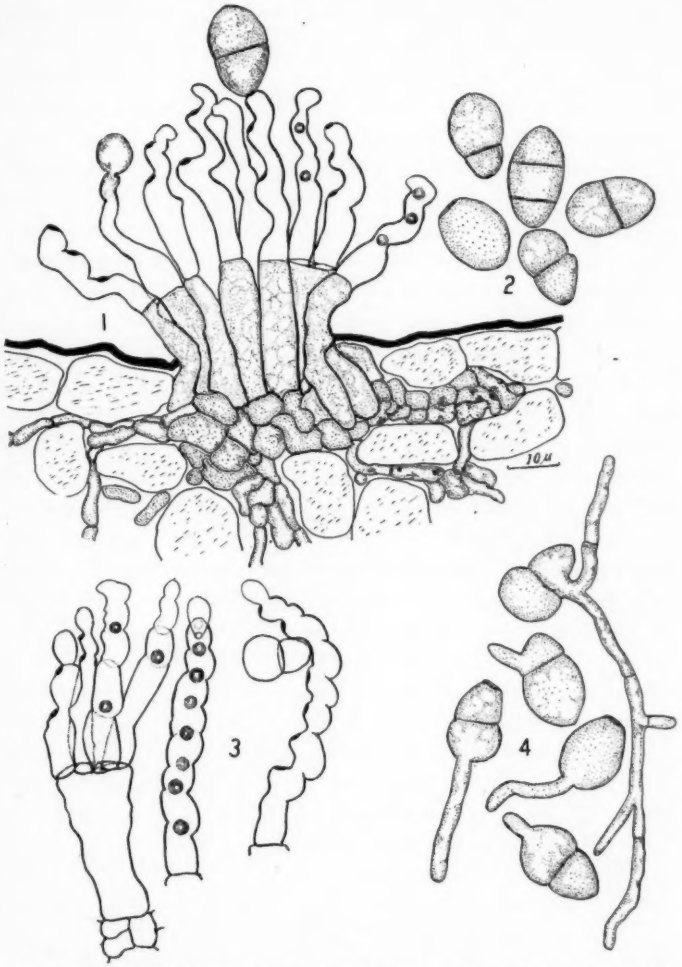

FIG. 4. 1, Conidial pustule in section showing the intercellular stroma and the foot-like cells surmounted by conidiophores. Scars from which the conidia have been detached are present; 2, Group of conidia. Most conidia are 2-celled although occasional ones are 1-celled or 3-celled; 3, Foot-like cell from a conidial pustule and conidiophores, a group of 4 conidiophores borne on one foot-cell, at the left; a row of 8 conidial scars and a young conidium being pushed aside by the elongating conidiophore, in the central figure; profile of a conidiophore that has borne 7 conidia, one still laterally attached, at the right.

abundant and when this material was stored out of doors for the winter, after it had remained in the laboratory for several weeks.

The trichogynes are collapsed by the time that the basal cells of the archicarps have become binucleate (FIG. 5, 6), indicating that the association of nuclei preparatory to fusion has occurred. The writer has not been able, however, to trace nuclear migration from the spermatia to the oögonial cells. Apparently all of the archicarps, except one, in each perithecial locule disintegrate. As this one continues its development its basal cells become multinucleate with pairs of closely associated nuclei (FIG. 5, 9).

A period of 4 to 6 months is required for the development of this portion of the perithecial structure. The subsequent development, including the swelling of the perithecial locule so that it protrudes above the stroma, the formation of a few to about 20 ascogenous hyphae in each perithecium, the fusion of the paired nuclei within the young ascus, the delimitation of the ascospores, and the rupture of the apex of the perithecium, may require from 4 to 6 weeks. The asci mature a few at a time, beginning with those nearest the center of the perithecium (FIG. 5, 10). The ascospores are at first elongate-elliptical and unicellular. They then become slender and septate, and at maturity are constricted at the septum and the upper cell is slightly the larger. Much of the increase in volume of the ascospores occurs in a brief period prior to their discharge (FIG. 5, 11). Expulsion normally occurs soon after maturity, as has been demonstrated by the presence of ascospores on agar plates inverted above perithecia. During germination on agar the spores first become much swollen and then a single germ tube is emitted from either cell or from both cells (FIG. 5, 12).

No portion of this fungus appears to be entirely hyaline. The stromata are brown to sepia, the cells of the perithecial locules are dilute honey yellow, the content of the asci before spore formation is honey yellow, and the ascospores are dilutedly honey yellow. Killian (**5**) and Bayliss-Elliott and Stanfield (**1**) regarded the ascospores as hyaline.

DISCUSSION

Studies on the developmental histories of representative genera of the Dothidiales, as yet, are distinctly limited in number. Almost nothing is known regarding the initiation of perithecia and sexuality in this order. A system of classification, based upon a definite knowledge of relationships within this order, can not be devised until such studies have been made. The monographic treatment by Theissen and Sydow (**7**) is indispensable to any student of the Dothidiales, and assuredly it must be revised in the light of future investigations. The basic concepts that have been established by the morphologic studies of Orton (**6**) emphasize the close relationship of the stromatoid Sphaeriales with the Dothidiales.

If one attempts to classify the fungus under consideration according to Theissen and Sydow's keys, it is plainly one of the family Dothidiaceae. Then because of the fact that the ascospores are pigmented, it will be found to resemble most closely the genus Systrema, erected by Theissen and Sydow (**7**). The only member of this genus whose ontogenetic development has been investigated is *Systrema Ulmi* (Schleich.) Theiss. & Syd. Killian (**4**) found, in this organism, that one celled conidia are abstricted from palisade-like cells at the surface of the stromata. He found within the young stroma what he interpreted to be a male and a female cell whose nuclei became associated by the migration of the male nuclei to the cell containing the female nuclei. Then on entering the ascogenous hyphae these pairs of nuclei united. This type of development is most certainly very different from that exhibited by the sooty blotch fungus of clover, and there seems to be little grounds for regarding it as of the same generic type. It should also be recalled that *Polythrincium* has remained for over a 100 years a monotypic genus [2] and that its characters are so distinctive that there appears little reason for disregarding them in classifying the pathogen on clovers. It is, therefore, proposed to erect for the clover fungus the new generic name *Cymadothea,* derived from words that refer to the undulate character of the conidio-

[2] Another species, *Polythrincium Shirainum* P. Henn. (Bot. Jahrbüch. Syst. Pflanzengesch. Pflanzengeog. **37**: 165, 1905) has been described as occurring, in Japan, on *Cercidophyllum japonicum* S. & Z.

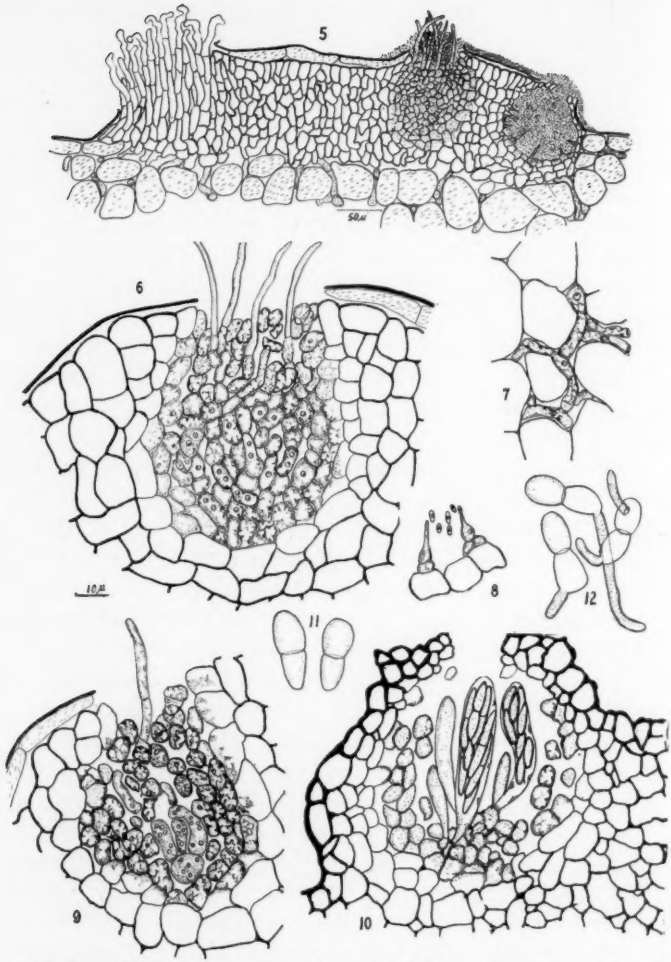

FIG. 5. 5, Section of a stroma showing, within one stroma, an old conidial pustule, a perithecial initial, and a spermogonium. The spermatia have welled out of the spermogonium and piled up among the projecting trichogynes; 6, Perithecial stroma in which the nuclei have become paired in the ascogones; 7, Intercellular hyphae within the tissue of green clover leaves; 8, Spermatiferous cells and spermatia from the parietal portion of the spermogonium; 9, Young perithecial stroma in which the ascogonium contains several pairs of nuclei that have arisen by conjugate division; 10, Perithecium of *Cymadothea Trifolii* that has irregularly opened and contains asci in different stages of maturity; 11, Mature ascospores immediately after expulsion onto agar plates; 12, Germinating ascospores.

phores of the conidial stage, and of the stromatic, dothidioid character of the perithecial stage.

Cymadothea gen. nov. (Etym. κῦμα, wavy or undulate, and δοθιήν, swelling or stromatoid outgrowth.)

Stroma innatum, erumpens, dothideoideum, brunneum; peritheciis in stromatibus oriundis, irregulariter dehiscentibus, sphericis vel ampulliformibus; ascis clavatis, aparaphysatis, octosporis; sporis didymis, subhyalinis vel helvis. Status conidicus *Polythrincium* est.

Cymadothea Trifolii (Pers.) comb. nov.

Syn. *Sphaeria Trifolii* Pers. Syn. Fung. 30. 1801.

Polythrincium Trifolii Schm. & Kze. Deutschl. Schwaemme **5**: 5. 1816.

Dothidea Trifolii (Pers.) Fries, Summa Veg. Scand. 387. 1849.

Phyllachora Trifolii (Pers.) Fuckel, Symb. Myc. 218. 1869.

Placosphaeria Trifolii (Pers.) Trav. Ann. Myc. **1**: 130. 1903.

Polythrincium Trifolii var. *platensis* Speg. Anal. Mus. Nac. Buenos Aires III. **13**: 437. 1911.

Phyllachora umbilicata Theiss. & Syd. Ann. Myc. **13**: 510. 1915.

Plowrightia Trifolii (Pers.) Killian, Rev. Path. Entom. Agr. **10**: 219. 1923.

Dothidella Trifolii (Pers.) Bayliss-Elliott & Stanfield, Trans. Brit. Myc. Soc. **9**: 226–227. 1924.

Stromaticibus pseudoparenchymaticis, brunneis vel atrofuscis; peritheciis in spermatiferis iisdem vel similibus evolutis, ampulliformibus; ascis clavatis, octosporis, aparaphysatis; sporis subhyalinis vel dilute helvis, 1-septatis, loculis inaequalibus, 20–26 × 8–9 μ.

Hab. in pagina adverso foliorum *Trifolii* emortui, in verno tempore.

Status spermogonicus: stromatibus in autumno efformantis, hypophyllis, pseudoparenchymaticis, ambitu irregulariter circularibus, brunneis v. atrofuscis, intus uni-pluri-loculatis, contextu loculorum parenchymatico et helvis; spermatiis ellipsoideis, saepe curvatis, 3–5 × 1.5–2.0 μ in guttulis vel cirris exsudatibus; spermatiophoris ampulliformibus. Hab. in foliis vivis v. dejectis *Trifolii*, socio *Polythrincio Trifolii*.

Status conidicus: Maculis fuscentibus; conidiophoris hypophyllis, fasciculatim erumpentibus, punctiformibus, deinde coalitis, ex mycelio in parenchymate folii oriundis, tortuosis vel exquisite undulatis, geniculatis, fuscis,

aseptatis, apice conidia gerentibus, posterius egredientibus et conidia alia gerentibus; conidiis ovatis, brunneolis, inaequaliter bicellularibus, inferiore cellula minor, 20–24 × 11–15 μ. Hab. in foliis viridibus *Trifolii* sps.

The microscopic preparations upon which this study has been based have been deposited in the Farlow Herbarium, Harvard University.

SUMMARY

This study deals with the morphology of the sooty blotch fungus of *Trifolium,* which has a conidial stage, a spermogonial stage, and a perithecial stage in its cycle of development.

The undulate character of the conidiophores is the result of sympodial branching. Each conidiophore produces a series of conidia. After each conidium is delimited the conidiophore elongates.

The spermogonia and perithecial fundaments are formed coincidentally within the same stroma, sometimes in separate stromata, in autumn. Each is locular.

The spermogonial stage functions in the production of spermatia that apparently are essential to the subsequent development of the perithecia.

The perithecial stage matures in spring within locules of a dothidiaceous stroma.

The evidence in hand indicates that all parts of the fungus are pigmented with some tint or shade of brown.

Several names have been applied to each of the stages in the developmental cycle of the organism. It is herein regarded as the type of a new genus and is given the name *Cymadothea Trifolii* (Pers.).

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A LITTLE KNOWN PECAN FUNGUS

VERA K. CHARLES

(WITH 2 TEXT FIGURES)

It frequently happens that a fungus will appear and be described and then be forgotten or pass unobserved for a long period of time. This appears to be the history of an interesting fungus recently observed on pecan leaves from Texas.[1] A search through the mycological collections of the Bureau of Plant Industry disclosed one specimen of this fungus collected in 1900 on pecan leaves, but erroneously determined as *Microstroma Juglandis* (Bereng.) Sacc. Another specimen in the Collections was collected by L. E. Miles in 1920 at DeSoto, Mississippi, on leaves of *Quercus* sp.

The fungus on pecan is very minute and appears as snow-white tufts on the lower surface of the leaves (TEXT FIG. 1, A).

A microscopic examination of the fungus on pecan showed it to be closely related to what Peck (**6**) described as the conidial stage of his *Ascomycetella quercina* (TEXT FIG. 2, B). The striking characters of the fungus are the tiered or fan-shaped, superimposed arrangement of the bundles of hyphae and the barrel-shaped cluster of closely adhering spores. Other details are given in the following description by Peck:

"Hyphae tufted, colorless, compound, composed of superimposed, somewhat obconic masses of obovate cells placed side by side and bearing on the upper and outer margin of the masses verticels of conidia; conidia oblong or subcylindrical, slightly curved, colorless, .0005–.0006 of an inch long, .00016–.0002 of an inch broad, produced in subelliptical tufts or masses, .0005–.0006 of an inch long and about .0005 of an inch broad, each tuft composed of seven occasionally six conidia, compactly placed side by side in a circle, and forming a cylinder around a central one." Peck, l. c.

[1] This material was received from J. B. Demaree in November, 1933, who collected it along the Colorado River at Utley, Texas.

Careful examination of Peck's material and that on the pecan leaves showed the group of spores to consist of eight instead of six spores. Peck's drawings also indicate this number.

Peck also described what he considered a new ascogenous genus, *Ascomycetella,* which was associated with this conidial form, and

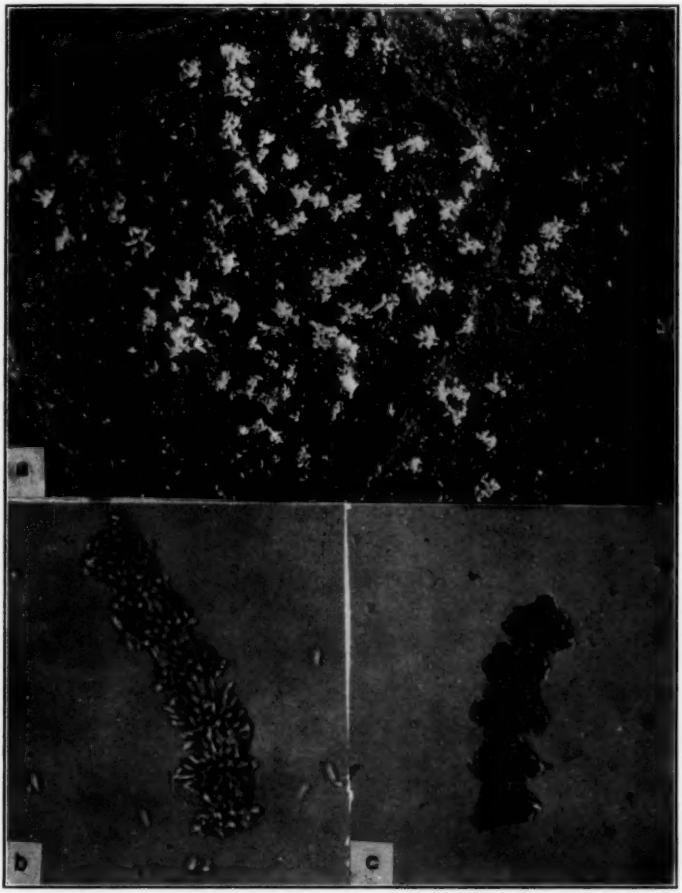

FIG. 1. a, Lower surface of a pecan leaf showing *Articularia quercina* (Peck) Höhn. var. *minor.* ×12; b, (From Desmaziere's Pl. Crypt. Fr., fasc. 19, no. *929,* 1838). ×425; c, *Articularia quercina* (Peck) Höhn. var. *minor.* ×425.

which he took to be its perfect stage. The genetic relationship of these two fungi was not demonstrated by culture work and the inference is that the supposed relationship was based only on the close association of the two fungi. No ascogenous fungus was found to be present on either of the two collections on pecans from Texas, the first in 1900 or the second in 1933.

The next study of this fungus seems to be that of von Höhnel (**3**) in 1909 who described a new genus to care for Peck's hypothetical conidial stage, and which he called *Articularia* (TEXT FIG. 2, D). The following is a translation of the description of this fungus as given by von Höhnel:

Articularia von Höhnel

Sterile hyphae consisting of a loose hyaline tubercular-like tissue. Fruiting hyphae simple, consisting of obconical tiers or sections, each tier composed of a whorl of obconical, truncate, single-celled conidiophores, each bearing a barrel-shaped cluster of 8 hyaline, parallel spores.

Von Höhnel assigned this genus to Mucedineae, but in concluding the discussion of the genus stated it might also be placed with the Tuberculariaceae.

In the same work von Höhnel (l. c.) described a second genus which he called *Articulariella* (TEXT FIG. 2, C) and which he considered to be the conidial stage of Ellis and Martin's (**2**) *Ascomycetella aurantiaca*, described in 1885. The following is a translation of the description of this genus and species as interpreted by von Höhnel:

Articulariella von Höhnel

Structure as in *Articularia* but the whorled side branches parallel with the axis, the ends enlarged or rounded and bearing a crown of 5–8 one-celled, oblong-elliptical spores which become separate.

A. aurantiaca (Ellis & Mart.) von Höhnel

Fungus white, sterile hyphae forming a tubercular tissue from which the long, fragile conidiophores arise and from which the asci later develop. The column of conidiophores consists of about 10–12 one-celled, hyaline, parallel hyphae, 20–40 × 2–3 μ in size, rounded at the apex and bearing a crown of 5–8 1-celled, elliptical-

oblong, hyaline spores, the latter straight, narrower at both ends and 6–9 × 1.5–2 μ in size.

Comparing this description with the original description by Ellis and Martin (l. c.), the general characters are found to be the same but the measurements of the spores are smaller, von Höhnel's measurements being 6–9 × 1.5–2 μ and those of Ellis and Martin 5–7 × 2–3 μ. The specimen of *A. aurantiaca* Ellis & Mart. examined during the course of this study was that of Ellis and Everhart, North American Fungi *2068*, 1883. An examination of this specimen showed the spore measurements to agree with those given by Ellis and Martin in their description of the fungus. In this instance, as in the case of *Articularia*, Peck's conidial stage, the genetic relationship was only inferred from the close association of the imperfect and ascogenous fungi and not demonstrated by culture work.

According to von Höhnel, *Articularia* differs from *Articulariella* in having closely adhering barrel-shaped bundles of cylindrical spores, eight in number, while in *Articulariella* the conidiophores are enlarged at the apex and bear a crown of 5–8 μ elongate, rod-shaped spores, 6–9 × 1.5–2 μ, with somewhat obtuse, narrower ends. However, as previously stated, a study of Ellis and Martin's material showed the spores to range from 6–7 μ in length. Von Höhnel considered this fungus his *Articulariella aurantiaca*, described by Ellis and Martin as the conidial stage of their *Ascomycetella aurantiaca*, to be the conidial stage of *Leptophyma aurantiaca* (Ellis & Martin) Sacc.

In discussing *Articularia* von Höhnel called attention to what he considered a nearly related fungus described by Corda as *Fusisporium uncigera*. In 1884, Saccardo (**7**) established the genus *Uncigera* for this fungus and called it *Uncigera Cordae* Sacc. Later, von Höhnel in discussing this species states that according to the rules of nomenclature it should be designated *Uncigera uncigerum* (Corda). The description and illustration of this specimen show it to have little resemblance to the fungus under consideration and may be eliminated from the discussion.

In 1925, sixteen years after von Höhnel's description of the two genera, *Articularia* and *Articulariella*, Arnaud (**1**) in his paper on "Les Asterinées" claimed that the two genera *Articularia* and

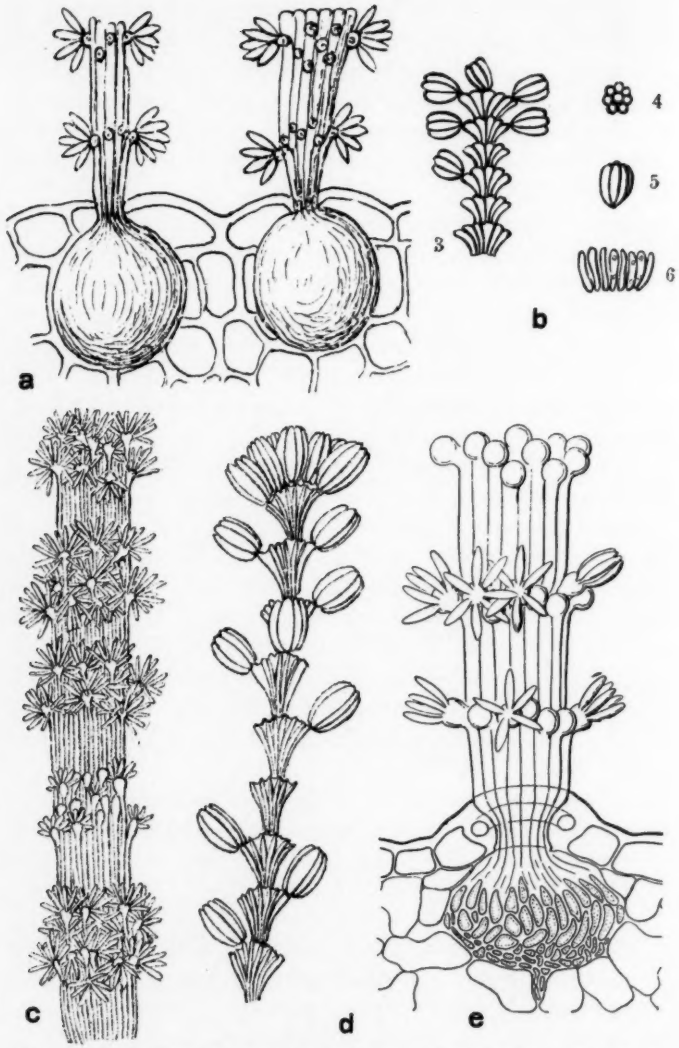

FIG. 2. a, *Helostroma album* (Desm.) Pat. After Patouillard; b, Conidial stage of *Ascomycetella quercina* Peck. After Peck. c, *Articulariella aurantiaca* (Ellis & Mart.) Höhn. After von Höhnel. d, *Articularia quercina* (Peck) Höhn. After von Höhnel. e, *Helostroma album* (Desm.) Pat. From Desmaziere's Pl. Crypt. Fr., fasc. 19, no. *929* (1838), under the name *Fusisporium album*. After Arnaud.

Articulariella are identical and referred them to *Helostroma album* (Desm.) Pat. (TEXT FIG. 2, A), a genus established by Patouillard (**5**) in 1902 to better describe *Fusisporium album* Desm.

The following is a free translation of Patouillard's description of *Helostroma* and shows a close agreement with von Höhnel's *Articulariella*:

Mycelium forming small, white, stromatic masses in the spongy parenchyma of the leaves, especially beneath the stroma. The tubercules consist of a filamentous structure about 25 μ and develop an erect, hyaline, cylindrical column emerging from the stroma, 25–35 $\times$ 7–10 μ, and truncate at the summit. The column is composed of colorless, parallel, cylindrical filaments 2–3 μ in diameter, crowded, unequally elongated and bearing the conidia at the apex on a short, lateral obtuse enlargement. The enlarged apices of the conidiophores appear as a crown about the column, sometimes a second crown develops towards the middle of the column. Six to seven, colorless, straight, elliptical conidia, measuring 5–6 $\times$ 2–3 μ, are borne on each conidiophore. Patouillard stated that Saccardo transferred this species to the genus Microstroma, near *Microstroma Juglandis* but from Saccardo's description of the monosporous, clavate basidia, Patouillard inferred that Saccardo must have had an entirely different species. Maire (**4**) also remarks that Saccardo's specimen was considered monosporous because all but one spore had fallen away. An examination of Saccardo's material contained in the Mycological Collections showed it to be characteristic of *Helostroma album* (Desm.) Pat. A microscopic examination of specimens of well known exsiccati and miscellaneous specimens in the Mycological Collections of the Bureau of Plant Industry, labelled *Microstroma album* (Desm.) Sacc., showed them to agree closely with Patouillard's genus *Helostroma*. Patouillard reported this fungus as parasitic on leaves of various oaks.

From the description and illustration of *Helostroma* by Patouillard and an examination of Desmaziere's material, it would seem that *Articulariella* von Höhnel is synonymous with *Helostroma album* (Desm.) Pat. Although von Höhnel's illustrations certainly show remarkable differences between the two fungi, Arnaud (l. c.) says that the apparent differences in the illustrations are

due to the fact that one figure was described from a surface view and the other a lateral view. This author made no mention of the difference in the shape of the conidiophores or in the size and shape of the spores. The article is accompanied by a plate showing a fragment of a leaf of *Quercus* from Rabenhorst-Winter, Fungi Europæi *3040,* which is Peck's material. The illustrations show only the detached agglutinated spores of Peck's so-called conidial form of *Ascomycetella quercina* or von Höhnel's *Articularia.* Arnaud gives no description nor illustration of the conidiophores or mention of the difference in the size and shape of the conidia. Probably Arnaud's specimen of Peck's material did not show the spores attached. The illustration shows the conidial form in close association with the ascogenous form, termed by Peck *Ascomycetella quercina.* In addition to Arnaud's plate there is a drawing of considerably larger magnification of *Fusisporium album* Desm. (TEXT FIG. 2, E) (Desmaziere's Pl. Crypt. Fr., fasc. 19, *No. 929,* 1838). This drawing agrees with our observations and interpretation of Desmaziere's material, but we can not consider it identical with Peck's material, that is his so-called conidial stage of *Ascomycetella quercina* or with our fungus on pecan. However, it does agree with our understanding of Patouillard's *Helostroma album* (Desm.), the fungus commonly known as *Microstroma album* (Desm.) Sacc. It appears that Saccardo's use of the generic name *Microstroma* has been followed by many mycologists although the generic characters of the genus do not satisfactorily describe this species.

During the present study many specimens of *Microstroma album* in well known sets of exsiccati and miscellaneous collections were examined and found to be typical of Desmaziere's *Fusisporium album.*

Arnaud further stated that *Articularia* and *Articulariella* do not represent the conidial stage of the ascogenous form, *Ascomycetella quercina,* described by Peck, but are two different fungi and that the ascogenous form is *Cookella microscopica* Sacc. present as a parasite on the *Helostroma.*

In view of the fact that there is a question of the genetic relationship of this fungus with an ascogenous form and without opportunity for field observations, it seems best to retain von

Höhnel's generic name, *Articularia,* until the life history of the fungus is definitely known. That this fungus may be classed with the Mucedineae as suggested by von Höhnel seems logical although it has characters which suggest the Tuberculariaceae, a reference also suggested by von Höhnel.

The material of *Articularia quercina* (Peck) von Höhnel available for study consisted of five specimens collected by F. S. Earle at Cobden, Ill., in 1882 and 1883 on *Quercus tinctoria,* a collection of G. H. Demetrio on *Carya alba* collected at Perryville, Mo., September, 1883, and issued by Rabenhorst-Winter (Fungi Eu. *3388b*) as *Microstroma leucosporum* (Mont.), and another collection by L. E. Miles at De Sota, Miss., Sept. 21, 1920 on *Quercus* sp.

The two fungi, the one described on oak, *Articularia quercina* (Peck) von Höhnel, and the form on pecan, are very similar in their general morphology but the spore measurements are sufficiently different to deserve a varietal name, accordingly the fungus on pecan will be designated *Articularia quercina* var. *minor* with the following description:

Articularia quercina (Peck) von Höhnel var **minor** var. nov.

Caespitulis candidis, flocculosis; conidiophoris 0.5 mm. alt., articulis 12–16 μ; conidiis oblongis-fusoideis, hyalinis, octonis in corpus ellipticodoliiforme 6–8 $\times$ 2.5–3 μ coalescentibus.

Fungus caespitose, effuse, white, hyphae forming closely adhering parallel columns, spuriously stilbaceous, compact superimposed, composed of obovate cells, placed side by side and bearing on the upper and outer margins 8 spores; spores oblong to subcylindrical, slightly curved, colorless, 6–8 $\times$ 2.5–3 μ, agglutinated in bundles.

Habitat on the lower surface of living leaves of *Hicoria illinoensis.*

Type: Utley, Texas, collected by J. B. Demaree, 1933. Deposited in Mycological Collections, Bureau of Plant Industry. A second collection by R. H. Price at College Station, Texas, Sept. 12, 1900.

SUMMARY

From the present study of the fungi, variously known as *Fusisporium album* Desm., *Helostroma album* (Desm.) Pat., *Articu-*

laria quercina (Peck) von Höhnel, *Articulariella aurantiaca* (Ellis & Martin) von Höhnel, *Ascomycetella quercina* Peck, *A. aurantiaca* Ellis & Martin, *Microstroma alba* (Desm.) Sacc., it is believed that *Articulariella aurantiaca* (Ellis & Martin) von Höhnel is synonymous with *Fusisporium album* Desm., *Helostroma album* (Desm.) Pat. and *Microstroma album* (Desm.) Sacc., but that *Articularia quercina* (Peck) von Höhnel is another fungus described as the conidial stage of Peck's *Ascomycetella quercina,* and in general, the fungus present on pecans.

Because of the difference in the size of the fungus on oak and the one on pecan, it seems best to consider the form on pecan a variety and designate it *Articularia quercina* (Peck) von Höhnel var. *minor.*

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NOTES AND BRIEF ARTICLES

An Unwelcome Guest

The May number of the *Gardeners' Chronicle* for 1934 contains an article by E. Holmes-Smith, Advisory Mycologist, North-Western Province, Manchester University, England, on "A playing-fields fungus menace." He states that large sums of money had been spent by the National Playing Fields Association to convert areas which had formerly been dumping grounds for all kinds of debris into playing fields. Tennis courts constructed on such grounds have been entirely ruined through the upheaval of certain fungi, apparently *Coprinus comatus,* which has insisted on intruding itself over the entire field in huge clumps.

Fig. 1. Tennis court destroyed by Coprinus.

Since the article was published as a warning it seems not out of place to repeat the warning here. In doing so the writer is in-

debted to E. Holmes-Smith for an original photograph of the ruined area which is reproduced here with his permission.

In MYCOLOGIA **25**: 150, 1933, a note was published by A. R. Bechtel on the "Lifting power of a mushroom." Keeping this in mind we may have some idea of the amount of damage that may be done by the eruption of a tennis court through the agency of these organisms.—F. J. SEAVER.

ARE LIVING SPORES TO BE FOUND OVER THE OCEAN?

Microörganisms are known to be rare over high mountains or large bodies of water. The writer, however, has not noted reports of exposure of Petri plates from a ship crossing the ocean. Such exposures were made from the SS. Alaunia, which left Montreal about noon May 25, 1934, and sailed *via* the St. Lawrence route south of Newfoundland to Plymouth, Havre and London. London was reached at noon June 4. The plates were exposed morning and evening, by holding them into the wind by hand. During the first three and the last two days of the trip, land was in sight and exposures of one or two minutes were made. During the intervening five days over the ocean, the exposures were for 2½ or 3 minutes. Eighteen plates in all were used, half being of Czapek's agar, half of potato-sucrose. The plates were then taken to the Imperial Mycological Institute, Kew, where they were incubated further and examined. The results showed that the precautions taken to avoid contamination were effective.

In the St. Lawrence River and Gulf, three or four microörganisms, about equally fungi and bacteria, were obtained on each exposure of two minutes. Cape Race, Newfoundland, was passed in a fog, and twenty bacteria developed from a 2½ minute exposure there at 8 A.M., May 28. With the last land astern, no microorganism was obtained for 4½ days, except for 3 colonies of bacteria on one plate exposed in mid-ocean on May 30; these perhaps were carried on an eddy of air from the ship. On June 1st at 8:45 P.M. the ship was just S. W. of Ireland with a wind blowing from the N. E.: 19 colonies of bacteria developed. The following morning an exposure south of the Irish coast yielded four fungi, one of which was *Botrytis cinerea* and another, appropriately enough, seemed to be *Phoma hibernica*. In the English

Channel 2 to 5 each of fungi and bacteria were obtained on each exposure of one minute, except for one blank in mid-channel.

The results obtained convinced the writer that microörganisms are so scarce over the ocean as to necessitate special arrangements for long exposures of plates and slides.—G. R. BISBY.

MUSHROOM POISONING DUE TO AMANITA COTHURNATA

In Ontario from June to September, 1934, the weather was drier and hotter than usual. Early to mid-September rains brought on a good crop of delayed fleshy fungi.

Amanita cothurnata Atk. was one species that appeared more plentiful than usual. On a ridge between two ravines, in a thin woods chiefly of beech, I observed a "fairy ring" of this agaric 18 feet in diameter. The plants were strictly limited to the circumference of the circle and along this line I counted 65 of them. The general appearance of the ring was white. The cuticle of the different pilei showed a range of color from very pale cream to apricot buff (R.); every part below it was pure white. One cap had become inverted without cracking and retained what must have been about a tea-cupful of rain water. I brought home the doubtfully largest specimen for further study. It measured 26 cm. over the marginally striated cap; the hollow stem reached 20.2 cm. above the base of the volva and bore a correspondingly ample annulus; the volva with a circumference of 18 cm. terminated above in a shallow but well marked, flaring roll. The flesh was odorless and the taste pleasant enough for a raw mushroom.

Lepiota naucina Fries was also plentiful and under the somewhat dangerous name "white mushrooms" was offered for sale on the market.

On Sunday, Oct. 7th, George Magorka of London, Ont., collected what he called "brown mushrooms"—*Hypholoma sublateritium*—and "white mushrooms"—*Amanita cothurnata*—in quantities to eat and to dry for winter use. In the evening about an equal division of the two kinds was prepared and boiled from 6 to 9 P.M., then seasoned, fried in cream, eaten and considered delicious. Supper over, the family consisting of the parents and a daughter retired in comfort for the night. But in the words of a poet "midnight saw another sight"; in less than three hours

they were all seized with serious illness. The father's irregular attempts to run to and from a physician's office attracted the attention of a policeman who for a time badly misjudged the situation. The doctor quickly responded to the call and assisted nature's efforts to get rid of the poison with warm mustard draughts and apomorphine and as quickly as possible had the patients removed to the hospital. There stomach irrigation, atropin injection and castor-oil purgation completed the treatment. Recovery was rapid.

In comparison with muscaria poisoning which it resembled in several respects there was no noticeable pupillary contraction or salivation. Promptitude of treatment should not be overlooked in reaching conclusions respecting the virulence of the toxicity of the cothurnate Amanita.

It was convenient for me to explore the locality of the collections and to examine the uncooked material. There is no reason to doubt that the two species named were the only ones involved. The family on feeling ill immediately blamed the "white mushrooms" because they had several times previously prepared and eaten the brown ones with no ill effects.—JOHN DEARNESS.

AN ADIRONDACK MYXOMYCETE

During the foray of the Mycological Society of America at Seventh Lake, Adirondack Mountains, New York, from August 21 to 24, 1934, Mr. Rispaud, associate of the writer, collected a Myxomycete of the genus *Lamproderma,* that later study would not reconcile with any species now recognized in the genus. A portion of the collection was sent to Miss G. Lister in England, who writes that it is similar to the type of *Enerthenema muscorum* Léveillé from New Granada (B. M. *1032*), and referred to in the Monograph, ed. 3, p. 154, where it was provisionally placed with *Lamproderma scintillans* (Berk. & Br.) Morgan; and that it seems to be a persistent form.

The Adirondack specimens are somewhat like *L. scintillans,* but the stalks are shorter and stouter, and the color of the sporangia is darker when the walls are removed. The capillitium is dark throughout, not pale at the base as in *L. scintillans.* The spores, 10 to 12 μ in diameter, are beyond the range of the latter species

in size, and are coarsely marked with dark, strong spines. It is not *L. scintillans,* nor any other recognized *Lamproderma,* and is evidently Léveillé's form which should be regarded as a distinct species.

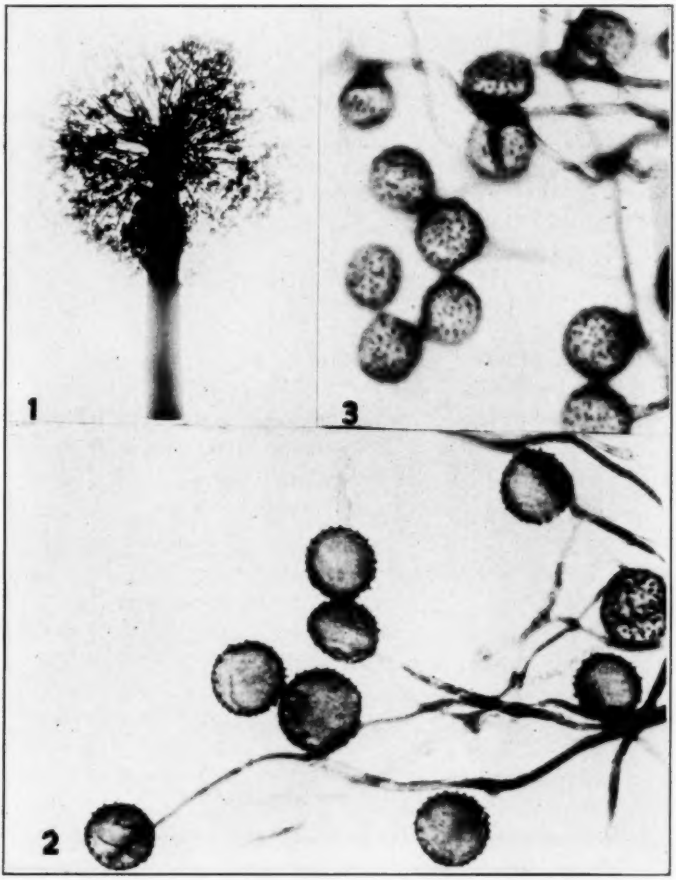

FIG. 1–3. *Lamproderma muscorum.* 1, Capillitium, × 100; 2, Spores, focussed on periphery, × 1000; 3, Spores, focussed on top, × 1000.

My thanks are due to Miss Lister for her aid and advice in relation to this form.

Lamproderma muscorum (Lév.) Hagelstein, comb. nov.

Enerthenema muscorum Lév. Ann. Sci. Nat. IV. **20**: 289. 1863.

Plasmodium? Total height .6 to 1. mm. Sporangia scattered, globose, stalked, erect, 0.3 to 0.5 mm. diam., blue or bronze, iridescent; sporangial wall thin, membraneous, somewhat persistent at the base. Stalk subulate-setaceous, black, shining, about one-half the total height, rising from a circular, purple-brown hypothallus. Columella thick, tapering slightly to the obtuse end, extending half-way into the sporangium. Capillitium dense, of rigid threads, radiating in all directions from the apex of the columella, dichotomously forking and branching, purple-brown throughout from base to tips. Spores violet-brown, 10 to 12 μ diam., marked with large, sharp, scattered spines.

On leaves. North of Seventh Lake, Adirondack Mountains, New York. August 24, 1934.—ROBERT HAGELSTEIN.

MINEOLA, N. Y.

In a previous issue of Mycologia (**25** (5) Sept.–Oct. 1933) under the heading of "Abnormal spores of some Ganoderma" I recorded the presence of "gasterospores" in some few specimens of *Ganoderma lucidum* and *Ganoderma applanatum*. Recently, in September and October 1934, I had the opportunity of subjecting four living specimens of *Ganoderma lucidum* attached to their substrata (pieces of *stem-bark and roots*) to continuous showers of running water from a tap in the laboratory for a week on different dates. In each case it was found that "gasterospores" were formed abundantly within the pore-tubes. I wonder if this might explain how "gasterospores" are formed in nature in those specimens which are directly exposed to continuous showers of rain for successive days.—S. R. BOSE

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